Applicability and Feasibility of Incorporating Microbial Fuel Cell Technology into Implantable Biomedical Devices

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Abstract

Microbial fuel cells (MFCs) have been shown to efficiently generate electricity from organic compounds and to serve as a method for waste treatment. Another possible application for this technology that has currently gained little attention is to generate power within the human body. Numerous implanted biomedical devices require power that is generally supplied by batteries with a finite life, necessitating another surgery to replace the power source. A method for continuously generating electricity within the body would revolutionize biomedical devices and enhance patient care. This paper examines the feasibility of applying microbial fuel cell technology to the areas of glucose sensing and cardiac pacing and discusses the problems that will likely be encountered. Due to the large number of variables that impact MFC power output, this technology is not likely to replace enzymatic glucose sensors currently in use. However, a well designed system implanted into the large intestines could provide adequate power for cardiac pacing, operate in continuous flow, utilize the natural flora of microbes, and take up less than 10% of the length of the large intestine.

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Introduction

Microbial fuel cells (MFCs) have demonstrated the ability to facilitate direct electron transfer from organic substrates to electrodes, producing a continuous electricity source that could be utilized for a variety of purposes. At the same time, implanted biomedical devices operating on chemical batteries often require additional surgeries to replace their battery after the chemical potential has been depleted. Considering that humans and microorganisms already have a symbiotic relationship, it may be possible to use MFC technology to solve the power source problem of implanted biomedical devices. MFCs can operate on a variety of substrates, and the glucose found in the bloodstream could produce electricity in an MFC in the same way that it fuels the metabolic reactions in the cells of the body. At the same time, if the power output generated by a glucose-operated MFC could be tightly correlated to glucose concentration, it would provide a new long-term option of glucose sensing for millions of diabetic patients worldwide. This paper will examine the possibility of utilizing MFC technology for cardiac pacing and glucose sensing. Thus, it is first imperative to understand the fundamentals of each of these areas.

Literature Review

Fuel Cells

Fuel cells are devices that generate electricity through oxidation-reduction chemistry. The fuel cell is composed of an anode and cathode. The anode is the site where a substrate, the fuel, is oxidized. Oxidation refers to the loss of electrons, and electrons that are released from this substrate are passed onto the anode in the fuel cell. The electrons travel into the cathode by way of electrical connections. The cathode is the site where reduction, the gain of electrons, occurs to the oxidant. Positive ions generated in the anode compartment travel into the cathode generally by way of an ion permeable membrane which completes the electrical circuit. Figure 1 below illustrates the electrochemical processes of a simple hydrogen fuel cell.

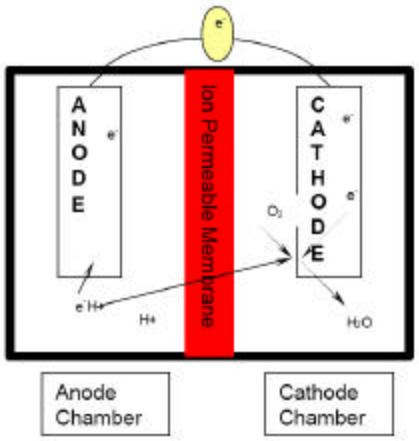

Figure 1: Hydrogen Fuel Cell (Henslee et al., 2004)

In this example, diatomic hydrogen gas is split at the anode releasing electrons according to the following equation.

$$H_2 \rightarrow 2H^+ + 2e^-$$

Equation 1: Oxidation of Hydrogen

These electrons are passed into the cathode compartment where they are transferred onto oxygen. This oxygen radical reacts with the protons generated in the anode that have now diffused through the ion permeable membrane forming water according to Equation 2.

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$$

Equation 2: Reduction of Oxygen to Water

This process produces a steady current through the wire connecting the anode and cathode. This electrical energy can be harnessed and made to do work.

Microbial Fuel Cells

Microorganisms have been utilized for some time in the production of electricity, but mostly in methanogenic digesters and biological hydrogen production (Angenenet et al., 2004). These methods rely on microbes to break down organic materials through fermentation which forms other combustible organic compounds such as methane and hydrogen. These compounds are then burned to produce energy needed to drive turbines for electricity production, such as in Equation 3.

$$CH_4 + O_2 \rightarrow CO_2 + 2H_2O + \text{heat}$$

Equation 3: Combustion of Methane

Non-combustible methods of producing electricity from microorganisms and organic substrates also exist through the use of Microbial Fuel Cells (MFCs). MFCs are a form of fuel cell that utilizes the metabolic processes of microorganisms to generate electrical energy from chemical energy (Allen and Bennetto, 1993). Like a standard fuel cell, an MFC has an anode and cathode compartment. Microbes in the anode compartment consume a substrate and oxidize it, releasing electrons in the process which end up on the surface of the anode (Jang et al., 2004). These electrons move into the cathode and are reduced as in a standard fuel cell discussed previously.

MFCs can be split into two categories; those that require a mediator and those that do not. Mediator microbial fuel cells use additional chemical catalysts to couple the transfer of electrons from the microbe to the electrode surface in the anode compartment. Most microorganisms are electrochemically inactive, and require the aid of mediators such as thionine or neutral red to operate in an MFC (Lithgow et al., 1986). The use of mediators can dramatically increase power output, although these mediators generally have a high cost, degrade rapidly, and may be toxic (Rabaey et al., 2005). These anode mediators have also been shown to be ineffective in a continuous flow system (Rabaey et al., 2005) Figure 2 below shows the use of a mediator (neutral red in this case) to assist in the harnessing of electrons from the oxidation of acetate by *E. coli*.

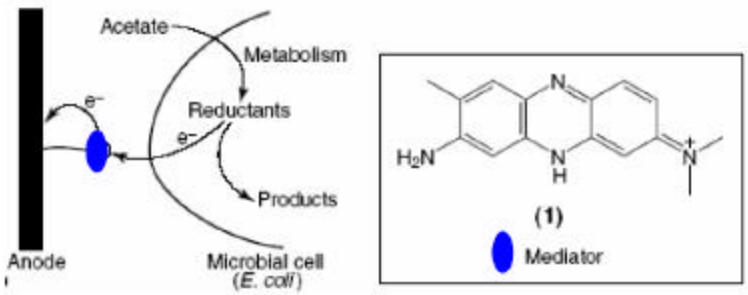

Figure 2: Oxidation of Acetate by *E. Coli* Using Neutral Red Mediator (Gasteiger, 2003 cited by Henslee et al., 2004).

Mediatorless MFCs do not require the aid of the mediator as the microorganism used is able to directly transfer electrons to the surface of the electrode. Far fewer microbes are able to operate using direct electron transfer than when external mediators are employed. Direct electron transfer has been shown to provide more efficient electron transfer, with coulombic efficiencies over 80% (Bond and Lovley, 2002). Figure 3 below demonstrates a mediatorless MFC.

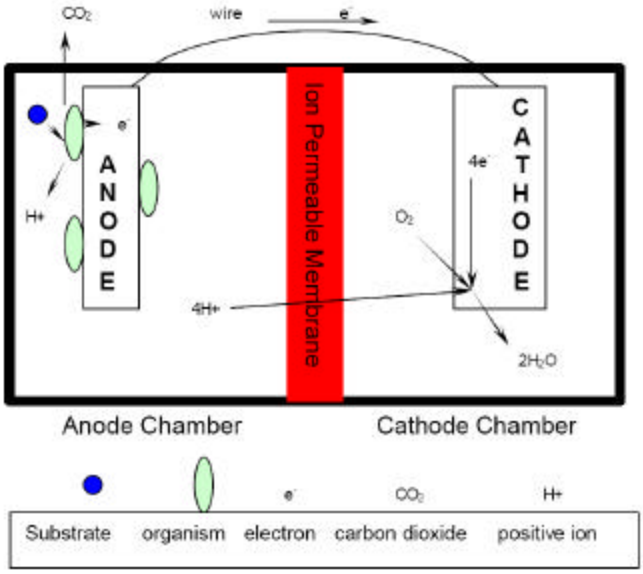

Figure 3: Mediatorless MFC (Henslee et al., 2004)

Microorganisms in MFCs

Some of the first microbes used in mediatorless MFCs were found in marine sediments (Bond and Lovley, 2002), and recent research has discovered microbe populations in municipal wastes that are suitable for mediatorless MFC application (Liu and Logan, 2004). The type of microbe to be used in a fuel cell depends on what substrate is intended to be oxidized. Different substrates also can produce different power outputs even if the same microbe is used, as demonstrated in Figure 4 below.

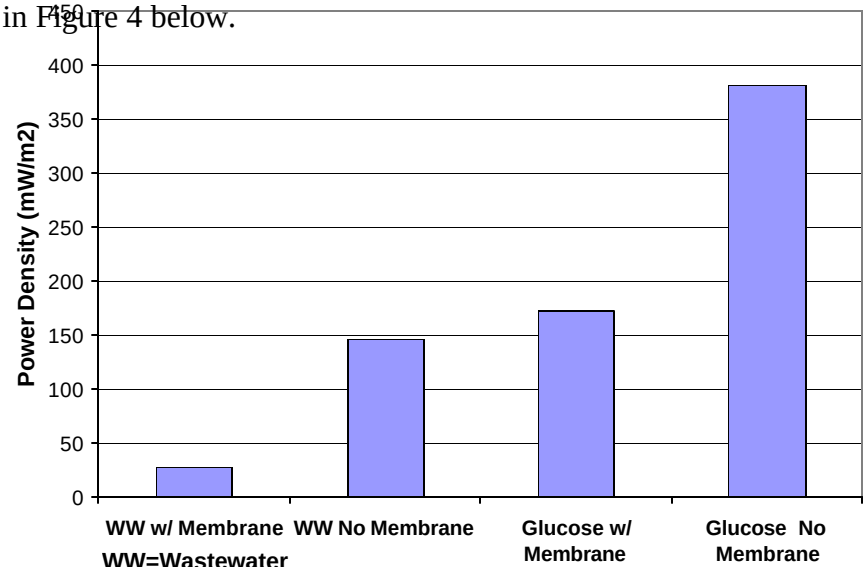

Figure 4: Effect of Substrate and Membrane on Power Density (modified from Liu and Logan, 2004)

Bacteria such as *Geobacter sulfurreducens* have been shown to operate on simple acetate but cannot handle more complex sugars (Bond and Lovley, 2002). Direct oxidation of glucose and fructose by *Rhodoferax ferrireducens* has been reported and provides a microbe that can be utilized in a wide range of possible applications (Chaudhuri and Lovley, 2003).

R. ferrireducens is a gram-negative facultative anaerobe shown to oxidize glucose with an 83% Coulombic efficiency. When operating a fuel cell, the current produced can be measured or calculated. Multiplying the amount of current transferred by the time in which this transfer occurred, the number of Coulombs transferred can be calculated. This is the actual yield of Coulombs obtained by the fuel cell. If one knows the theoretical yield of Coulombs, the Coulombic efficiency can be determined. The oxidation of glucose to carbon dioxide proceeds according to the Equation 4.

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$$

Equation 4: Oxidation of Glucose

From this equation it can be seen that one mol of glucose produces 24 mols of electrons. Since there are 96,500 Coulombs in one mol of electrons, the number of Coulombs theoretically available from one mol of glucose is 2.32×10^6.

Chaudhuri and Lovely demonstrated in 2003 that a fuel cell using *R. ferrireducens* showed the "ability to be recharged to its nearly original charged state following discharge, no severe capacity fading on charge/discharge cycling, the ability to accept fast recharge, reasonable cycle life, and low capacity loss in prolonged storage under idle conditions". This organism is quite versatile and may have the potential to be used in biomedical applications.

Design of MFCs

MFCs can be constructed in a manner that separates the anode and cathode into separate chambers (two chamber MFC) or in a manner where both are in one compartment (single chamber MFC). The figures below show examples of one and two chambered MFCs

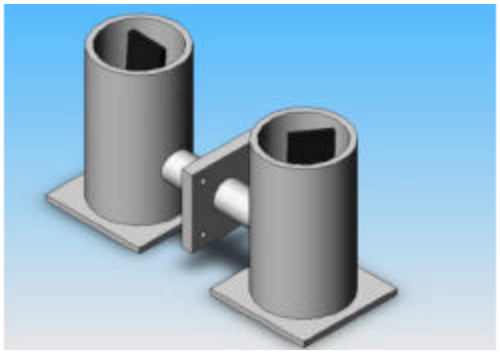

Figure 5: Two Chamber MFC

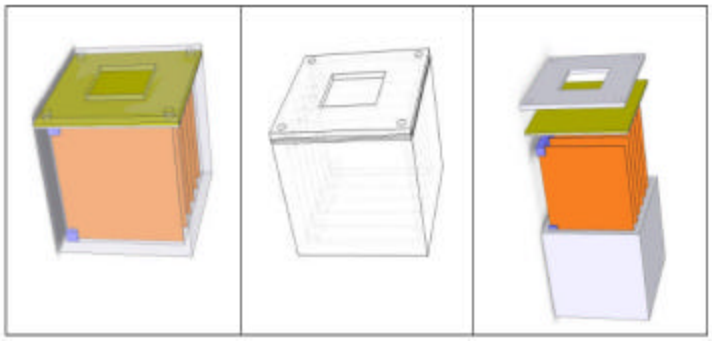

Figure 6: Single Chamber MFC

The primary difference between single and double chamber MFCs is the absence of an ion permeable membrane in the single chamber MFC. Both the anode and cathode are housed in the same compartment and are thus subjected to the same solutions and similar environments. The cathode is exposed to the solution in the chamber on one side and external air on the opposite side. In the double chamber MFC, different conditions (such as anaerobic, aerobic, etc.) can be maintained in each compartment. The rate of oxygen diffusion into the anode without an ion permeable membrane is 2.7 times higher than a double chamber design using a Nafion 117 membrane (Liu and Logan, 2004). The advantages of a single chamber MFC are reduced setup costs, due to the absence of expensive membranes (a 10cm x 10cm piece of Nafion 117 costs $31 (FuelCell Store, 2006), and higher power output. A disadvantage to the single chamber is a decrease in Coulombic efficiency generally attributed to the diffusion of oxygen to the anode area. Facultatively anaerobic bacteria will pass their electrons directly to oxygen rather than the surface of the electrode if oxygen is available, reducing the efficiency of the fuel cell. The figure below demonstrates the relationship between increased power density and decreased Coulombic efficiency due to the absence of the membrane.

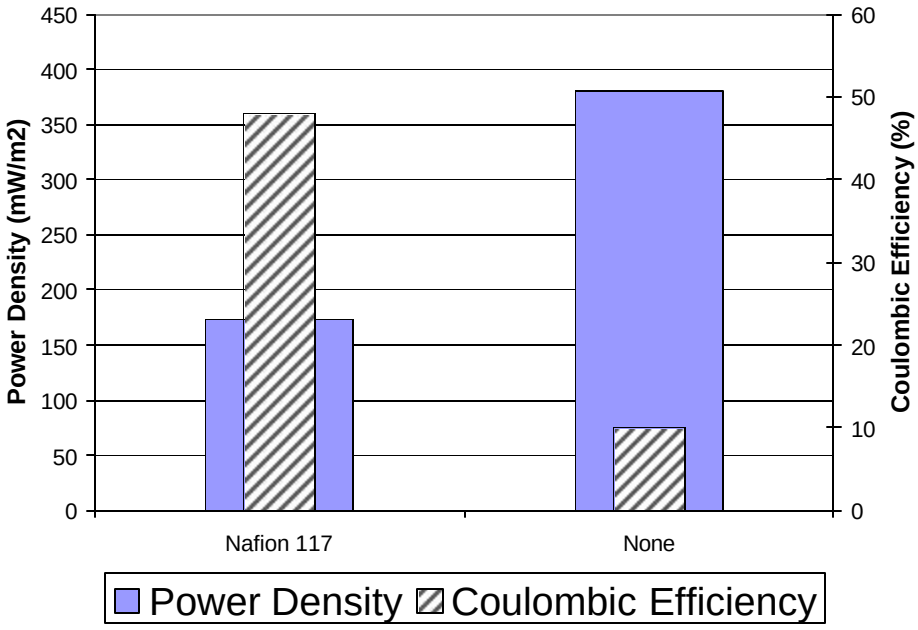

Figure 7: Effect of Membrane on Power Denisty (modified from Liu and Logan, 2004)

An interesting hybrid of the single and double chamber design was used by Tartakovsky and Guiot (2006). As shown in Figure 8 below, this upflow MFC has two chambers separated by a simple sponge rather than an ion permeable membrane. Influent is fed into the bottom (anode) and effluent is removed from the top (cathode). Ions and fluids can diffuse through the sponge, but the sponge provides some separation of the compartments allowing different environments to be maintained. In this case, the anode could be kept more anaerobic than the cathode and efficiency was increased.

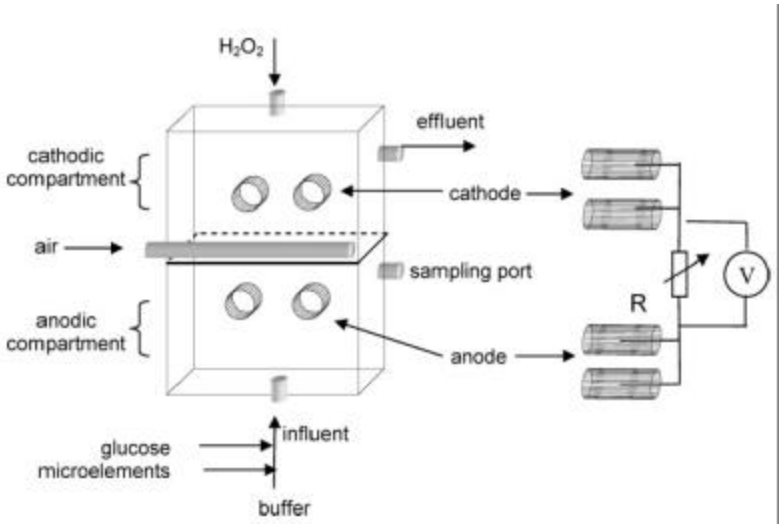

Figure 8: Continuous Upflow MFC (Tartakovsky and Guiot, 2006)

MFCs can be operated in batch or continuous modes. A batch mode involves setting up the MFC with the desired substrates and allowing it to run to completion, followed by the manual addition of more substrate. A continuous flow system uses pumps to add more substrate and remove wastes without manual interference. Rabaey et al. (2005) demonstrated that operating a non-mediated MFC in continuous mode rather than batch decreased power output from 479W/m^3 to 49W/m^3. Although it causes a decrease in power output, continuous flow MFCs have broader possible applications because they require less maintenance.

Electrodes in MFCs

The electrode materials used can have a significant impact on density. Anode electrodes are typically constructed out of different graphite materials including plate, paper, felt, and foam. The more porous materials generally have a higher power density due to more intricate surface area available for microbial colonization. Bond and Lovley (2002) demonstrated that the power output was strongly correlated to anode surface area. The cathode surface area, in contrast, has only a minor effect on power output (Logan et al., 2004). Figure 9 shows a comparison of three different graphite materials by Chaudhuri and Lovley (2003) with all variables except electrode materials held constant.

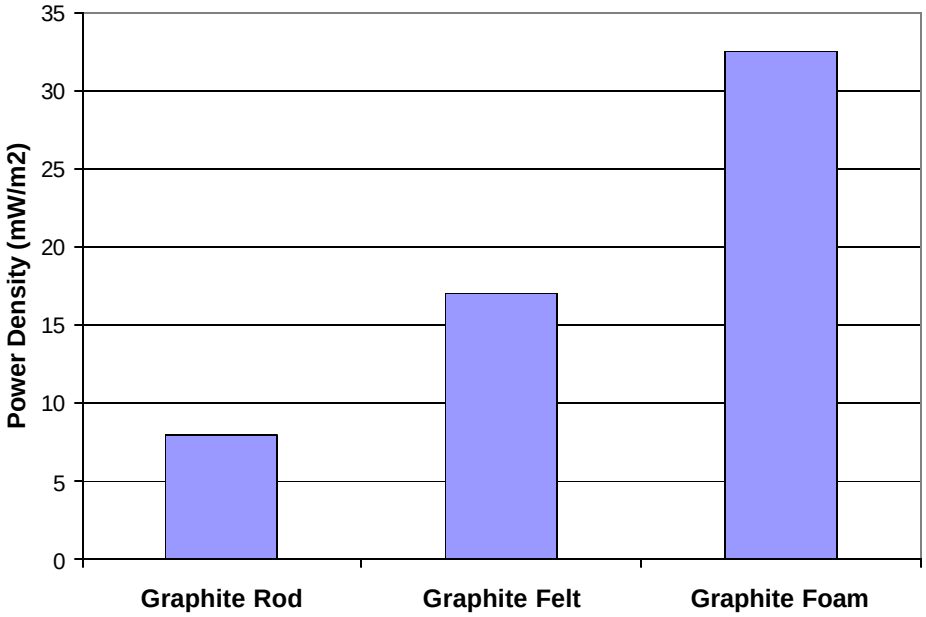

Figure 9: Effect of Electrode Materials on Power Density (modified from Chaudhuri and Lovley, 2003)

Previous work in our own lab has also demonstrated the effect of electrode materials on power output in addition to the difficulty in obtaining precision with multiple trials with MFCs, as shown in Figure 10 below.

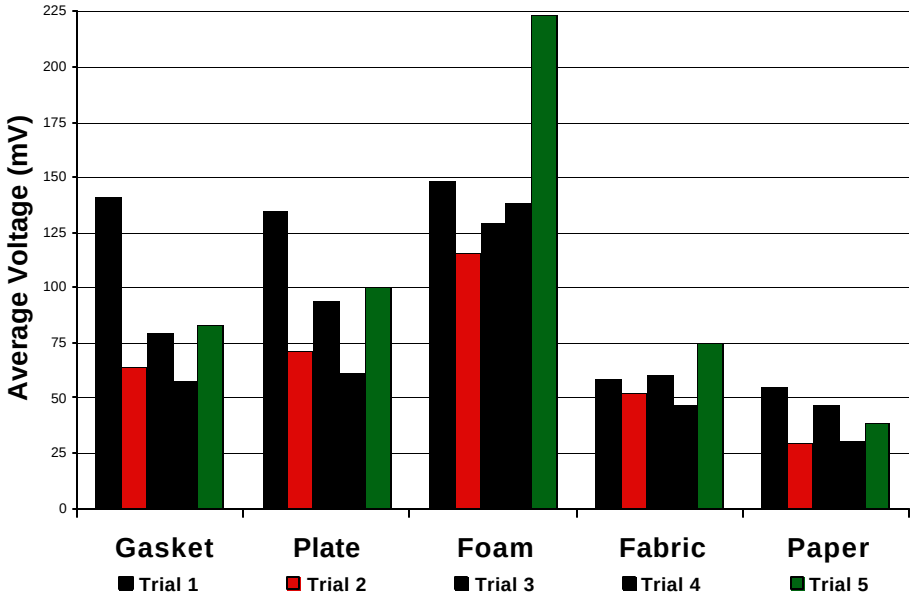

Figure 10: Effect of Electrode materials on Voltage in Dairy Wastewater MFCs (Henslee et al., 2005)

Incorporation of platinum catalysts into the cathode electrode significantly increases power output. Logan et al. (2004) demonstrated that a 78% reduction in power density occurs in the absence of platinum catalysts in the cathode. Mediators such as potassium ferricyanide can facilitate the transfer of electrons from the cathode to oxygen, although these mediators can be expensive, toxic, and degradable over time. These cathode mediators are also not applicable to a continuous flow system (Rabaey et al., 2005).

Effect of Resistance in MFCs

The microorganisms oxidizing the substrate release electrons onto the electrode surface and should thus be considered a current source. However, this current source is not constant, but is affected by the amount of resistance in the system. There is not a linear relationship between voltage and current in this case. The optimal resistance to be used in each setup depends on numerous factors, including the application, substrate, inoculum, type of reactor, and any

potential losses within the system (Rabaey et al., 2005). Figure 10 below shows an example of the varying relationship between resistance and power output.

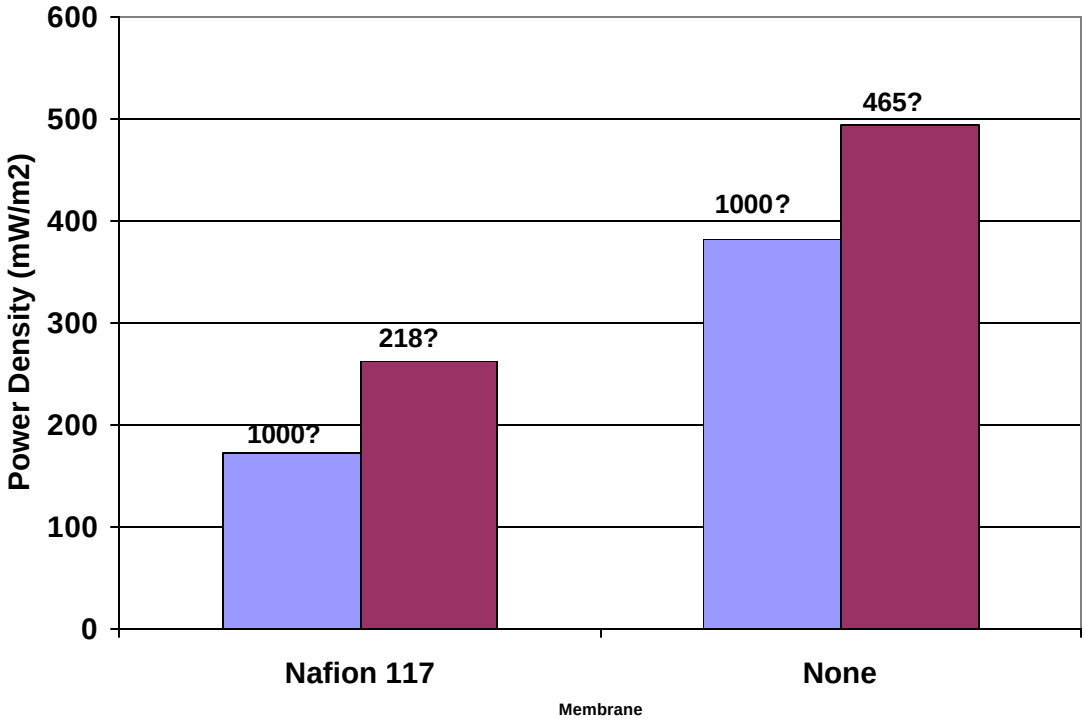

Figure 11: Resistance and Power Density (modified from Liu and Logan, 2004)

Since MFCs have been shown to operate on glucose, a biomedical device could theoretically be powered by an MFC using glucose in the body. It is thus important to first have a basic understanding of the role of glucose in the body, how glucose levels are determined, and of glucose disorders that could be treated with the aid of an MFC.

Glucose in the Human Body

Glucose is one of the primary fuels used by the human body to complete its metabolic requirements. The amount of glucose in the blood stream, denoted by the term 'blood sugar' or 'blood glucose', is in the range of 60-115mg/dL for a healthy individual. Normal blood sugar levels will be on the low end of that scale following a period of fasting, while immediately following a meal the blood glucose levels may rise up to 140mg/dL. It is crucial for the body to maintain at least 60mg/dL of blood glucose otherwise the body will enter a state of hypoglycemia. This state is quite dangerous as the brain requires glucose for adequate function.

Prolonged exposure to a hypoglycemic state can lead to dizziness, confusion, coma, and even death. If the blood sugar is too high, the body enters a state of hyperglycemia. Hyperglycemia, or elevated blood sugars, is the trademark sign if a disease known as diabetes mellitus.

Diabetes Mellitus

According to the American Diabetes Association (2006), over 18.2 million people in the United States have diabetes, and almost half of them are unaware of their disorder. Diabetes is a dangerous disorder that can be readily treated if a diagnosis is made early on. There is no cure for diabetes, and patients must continually be involved with their own treatment. Diabetes is diagnosed by having a fasting (at least ten hours following a meal) blood sugar level over 200mg/dL (Saudek et al., 1997). The cause of this elevated blood glucose level is a malfunction with the body's insulin or insulin producing cells. Insulin is a hormone that is secreted by the ß-cells on the islets of Langerhans in the pancreas. This hormone enters the blood stream and interacts with the cells of the body. By manipulating characteristics of the cell membrane, insulin allows glucose from the blood stream to enter cells. Without the presence of insulin, glucose will remain in the blood stream and may eventually be filtered into the urine by the kidneys. As shown in the figure below, blood glucose levels in diabetics significantly differ from the normal range.

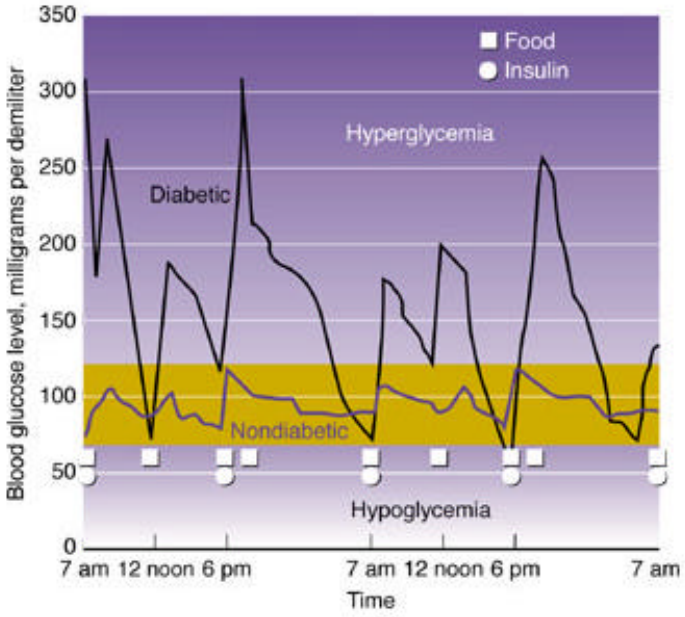

Figure 12: Blood Glucose Levels in Diabetics and Non-Diabetics (Saudek et al., 1997)

Immediately following a meal, the blood sugar levels in the body will be elevated due to the recent fuel intake. These elevated levels signal the pancreas to release insulin which allows the glucose to enter cells and reduces blood glucose levels. After the glucose levels have declined, the pancreas ceases to excrete insulin. At baseline blood glucose levels, the cells are utilizing other fuel sources, such as fats or stored carbohydrates, rather than relying on glucose in the bloodstream (Saudek et al., 1997).

There are multiple types of diabetes, each with their own causes and treatments. All forms, though, result in elevated blood sugar levels. These elevated sugar levels result from inadequate insulin, which may be caused by heredity, virus, resistance, drugs, or an autoimmune disorder (Saudek et al., 1997). Type I diabetes results from a total or near complete destruction of the ß-cells in the pancreas. This prevents the body from producing its own insulin. Treatment for Type I diabetes requires insulin supplementation, which is why Type I diabetes is known as "insulin dependent" diabetes. Type II diabetes results from the body not responding properly to the insulin produced by the pancreas. The main difference between Type I and II is that in type II, insulin is produced by the pancreas whereas this function is absent in Type I. In Type II, there simply may not be enough insulin produced or the body may have become desensitized to its own insulin. Over 90% of all diabetics have Type II diabetes (Saudek et al., 1997). This type has a strong correlation to obesity and is mostly seen in overweight patients over the age of 40. This is why Type II diabetes is frequently referred to as "Adult Onset" diabetes. Although insulin supplementation may be used as a form of treatment, Type II diabetes is also known as "Non-Insulin-Dependent" in contrast to Type I (Saudek et al., 1997). Regaining control of one's body weight can often cure the symptoms of Type II diabetes, thus these patients may not need insulin supplementation if altering their diet is sufficient.

Complications of Diabetes

There are numerous complications caused by diabetes that lower the quality of life for millions of people with this disease. Increased thirst and increased urination are caused by elevated blood sugar levels, as the blood becomes more like syrup when too much glucose is present. The brain triggers a sensation of thirst which causes the patient to drink more water. This causes the body to swell, which triggers the kidneys to eliminate the excess fluids consumed. Increased blood

sugar also causes the lens in the eyes to swell, which prevents them from bending as normal and causes blurred vision. Without insulin, the cells of the body are starved of glucose and begin to utilize other sources of energy such as fat and stored carbohydrates. This causes a feeling of fatigue as well as weight loss. When the body degrades fats, it produces ketones in the process. Through continued fat degradation, the blood will become saturated with ketones which will lower the pH of the blood. This condition is known as ketoacidosis and can cause a coma and be fatal. Elevated glucose levels provide an ideal situation for the growth of bacteria, in addition to slowing down the immune response (Saudek et al., 1997). This is why infections are more common and more difficult to treat in patients with diabetes. Prolonged exposure to elevated glucose levels may eventually lead to disorders such as arteriosclerosis, neuropathy, and nephropathy (Saudek et al., 1997).

Treatment of Diabetes

Treatment options for diabetes range from diet and weight control, to insulin supplementation, and even to pancreas or islets of Langerhans transplants (Shapiro et al., 2000). However, transplants require availability of organs in addition to long term use of immunosuppressant drugs, so the latter option of treatment is rarely used (Renard, 2002). Patients who are being treated for diabetes are required to test their blood sugar levels and to supplement their bodies with insulin if necessary. There are numerous methods available to test blood sugar levels (finger sticks, subcutaneous enzymatic sensors, implantable sensors) and to supply insulin to the body (injections as well as external and implantable insulin pumps). There has been a plethora of research into creating an artificial ß-cell that would be able to perform the functions of a physiological ß-cell. The three main elements of this device would be accurate glucose sensing, efficient insulin delivery, and a control system to correlate insulin delivery to actual and expected blood sugar levels (Renard, 2002).

Blood Glucose Sensing

The most important element of any method for determining blood sugar levels is accuracy. "Accuracy implies precision, linearity, sensitivity, selectivity, and stability of glucose measurement during *in vivo* use" (Renard, 2002). A method may actually measure electricity or a chemical other than glucose to indirectly determine blood sugar concentration, but it must

reproducibly correlate to the concentration of glucose in the blood stream no matter what chemical or current is actually measured. The value determined by the method should be close enough to the actual value that the patient's health is not jeopardized. It is important that the sensor response be sensitive to small changes in glucose levels in addition to avoiding interference from other chemicals in the blood stream. These methods must be stable enough for long term use so that a patient only needs to adapt to and master one method.

Finger Sticks

The traditional method of testing blood glucose levels involves utilizing a lancing device on the finger to draw out a drop of blood. This blood is put onto a test strip that is coated with an enzyme, usually glucose oxidase. This enzyme catalyzes the oxidation of glucose by the following formula (Renard, 2002):

$$\text{Glucose}(C_6H_{12}O_6) + O_2 \rightarrow \text{Gluconic acid}(C_6H_{12}O_7) + H_2O_2$$

Equation 5: Catalyzed Oxidation of Glucose

The hydrogen peroxide produced in this reaction dissociates:

$$H_2O_2 \rightarrow 2H^+ + O_2 + 2e^-$$

Equation 6: Hydrogen Peroxide Dissociation

The test strip will then change colors based on the amount of hydrogen ion produced, which can be correlated to glucose concentration (Saudek et al., 1997). In newer electronic testing devices, the current produced by the dissociation of peroxide is measured and correlated to glucose concentration, which is then displayed on the digital readout (Renard, 2002). This method of testing provides accurate blood glucose levels, but only at intermittent time periods. This method is painful for the patient and cannot provide real time continuous reading of blood glucose levels.

Optical Sensors

One noninvasive method for determining blood glucose levels is through optical sensing. A beam of light is shined onto the fingertip and the amount of light absorbed is measured. It has been determined that absorption of light in the range of 700-1300nm can be correlated to blood glucose concentration (Jaremko and Rorstad, 1998). There are several problems with this option,

though, in that other chemicals in the blood stream (such as oxygen) also absorb light and can alter the absorption pattern. This method has also been shown to not measure accurate glucose concentrations over long periods of time (Gough and Armour, 1995).

Reverse Iontophoresis

Another noninvasive method for determining glucose levels is through reverse iontophoresis. A small amount of current is applied directly to the surface of the skin and it causes glucose to surface from the interstitial fluid. The benefits of this option are that it has shown linearity in sensor signal to glucose concentration and the amount of current used can specifically withdraw glucose alone. Problems with this method include a lag time between sensor readings of interstitial fluid and actual blood glucose levels (Potts et al., 2002), difficulty in measuring hypoglycemic glucose levels (Pitzer et al., 2001), and eventual skin reactions at the site of current application (Gough et al, 1997). Thus, this method can only be used for short term applications and is not suitable for long term glucose sensing or as part of an artificial ß-cell.

Subcutaneous Enzymatic Glucose Sensors

Many glucose sensors measure the glucose concentration in the interstitial fluid of the dermal tissue and then correlate this to blood glucose concentrations. Interstitial fluid is one of the two main components of extracellular fluid; the other is plasma. The main difference between interstitial fluid and plasma is that plasma has a much higher concentration of proteins present. Endothelium of the capillaries is impervious to the proteins in plasma, but allows the small water soluble molecules of glucose to diffuse across the membrane (Best, 1989). The glucose concentrations of plasma and interstitial fluid will not necessarily be the same in all areas of the skin due to different metabolic variations at different locations (Gerritsen et al., 1999). While it is the interstitial fluid glucose concentrations that are being measured by subcutaneous sensors, it is the plasma glucose concentration that more accurately reflects the glucose concentration in the blood. There also exists a lag time between elevated blood glucose levels and a corresponding elevation of interstitial glucose levels of up to 45 minutes (Gerritsen et al., 1999). Even if the interstitial glucose concentration is measured precisely, it may not provide an accurate measurement of the blood glucose levels.

Three generations of subcutaneous enzymatic glucose sensors have been developed. Each generation is based on the principle of immobilizing the enzyme glucose oxidase onto an electrode. Glucose oxidase catalyzes the oxidation of glucose according to Equation 1 above. Following the oxidation of glucose to gluconic acid and hydrogen peroxide, the peroxide will decompose according to Equation 6. Glucose oxidase is highly specific for glucose, which enhances the accuracy of the device.

The first generation of these glucose biosensors actually measures either the amount of hydrogen peroxide produced or the amount of oxygen deficit created by glucose oxidation. The sensors are designed with a membrane containing bound glucose oxidase covering a platinum electrode (Gerritsen et al., 1999). Glucose and oxygen diffuse through this membrane and undergo catalyzed oxidation. The generated hydrogen peroxide diffuses to the electrode surface where it is oxidized and releases electrons onto the electrode surface. The current that is generated can be measured and correlated to the original amount of glucose present. This method has advantages of "easy fabrication and the possibility of constructing small sized sensors" (Wilson et al., 1992). The oxygen detecting method is very similar, except that the amount of oxygen consumed at the sensor site is compared to a reference electrode that does not contain glucose oxidase. The oxygen deficit is calculated and correlated to glucose concentration.

Second generation enzymatic sensors use the same method as first generation sensors but employ chemical mediators such as ferrocene to carry electrons from the enzyme to the electrode. The advantage of these sensors is that outside electrochemical interference is reduced (Wilson et al., 1992), although they have been shown to possibly leak the toxic mediator which may jeopardize the health of the patient (Wilkins and Atanasov, 1996). Third generation sensors eliminate the need for mediators by using direct electron transfer from the enzyme to the electrode. The enzymes are bound to the electrode surface so that no diffusion or use of mediators is required. Although these latter two generations of sensors are more accurate, clinical trials have predominantly utilized first generation sensors (Gerritsen et al., 1999).

Attempts have been made to utilize this technology for an implanted sensor rather than just for short term use. In vivo studies have demonstrated that these implanted sensors are only

functional for a matter of hours to several days (Gerritsen et al., 1999). Most of the complications that arise are due to biocompatibility issues and the host response to the sensor. The conditions encountered in the body are difficult to replicate in the laboratory. For example, one of the more promising sensors developed utilizing more glucose-selective membrane reported accuracy up to 11 days in vitro, but only 24 hours in vivo (Ward and Jansen, 2002). Biocompatibility issues will be discussed further in a later section. Even with an implanted sensor, patients are still required to draw blood samples and test blood glucose levels at least four times each day. These data are used to calibrate the implanted sensor as "almost all implanted glucose sensors generally show a significant drift in sensitivity during implantation" (Gerritsen et al., 1999).

Microdialysis

A modification of the enzymatic sensor has been developed that attempts to circumvent some of the failure mechanisms encountered by the implanted sensor. This method, known as microdialysis, places a hollow fiber underneath the skin that allows interstitial fluid to enter the tube. This fluid can be continuously withdrawn from the tube and tested in an external pager size unit that contains glucose oxidase (Bolinder et al., 1997). Although this eliminates some of the biocompatibility issues associated with long term implantation, problems encountered have included poor glucose recovery, significant lag times between interstitial glucose concentration and blood glucose concentration, and fiber obstruction (Rhemrev-Boom et al., 2002). When the device functions properly, it has shown excellent linearity between generated signal and glucose levels (Maran et al., 2002).

Insulin Supplementation

In order to achieve the goal of developing an artificial ß-cell, an efficient method of insulin supplementation is necessary. Since insulin is required at all times and in different quantities through the day, the ideal supplementation device would have continuous flow capabilities and be easily controllable. Current insulin infusion methods include injections or pumps, and the method of infusion may use either the subcutaneous or intraperitoneal route.

Insulin Injections

The primary method for insulin supplementation is through injections into the abdomen, arm, leg, or buttocks. A patient will inject a calculated dose (measured in Units) of insulin in the morning, evening, and/or prior to a meal. The dose is calculated by determining how much insulin will be needed to break down the food about to be ingested. This process has numerous opportunities for error, and patient compliance can be a problem. According to the American Diabetes Association (2006), the cost of the materials and insulin needed for injection runs around $1.50 per day.

Continuous Subcutaneous Insulin Infusion Pump

Continuous subcutaneous insulin infusion (CSII) is a process where insulin can be administered continuously throughout the day. A set amount of insulin is delivered at all times, and a larger set amount (a bolus) can be delivered prior to a meal. A patient will insert a needle or cannula under the skin of the abdomen. This cannula is connected to a pager-size external insulin pump that delivers the programmed amounts of insulin. The cannula is changed every 2-3 days, and no additional injections of insulin are necessary. This technology is now used as the ideal treatment for Type I diabetes and assists over 200,000 diabetic patients worldwide (Pickup and Keen, 2002). The benefits of this device are that it eliminates the need for painful insulin injections, provides the body with a basal amount of insulin at all times, and actually lowers the required amount of daily insulin by 14% on average (Pickup and Keen, 2002). However, if the device fails to function normally due to catheter blockage or other malfunction, ketoacidosis can develop rapidly (Selam and Charles, 1990). One of the main problems with CSII is that it relies on the body to absorb insulin that is injected under the skin. There is a significant lag time between when insulin is injected and when it will enter the blood stream. This can create a problem if a patient does not increase the pump output early enough before eating a meal, as blood sugars will skyrocket before the insulin can be delivered to the bloodstream. Likewise, it is difficult to remove excess insulin from the skin before it can be absorbed by the body. Thus, if too much insulin is administered too late, the blood sugars will plummet farther than desired due to the unanticipated lag time effect. This can cause severe hypoglycemia without warning. New types of insulin that have a shorter effective lifetime have been developed to reduce these problems (Renard, 2002). Patients who utilize the pump must be well trained to understand how

it works and how to adjust their insulin levels. Selecting appropriate patients is crucial to device success, and there are now well-accepted rules for determining which patients are appropriate (Pickup and Keen, 2002). According to the American Diabetes Association, the cost of a subcutaneous insulin pump is approximately $4,000-$6,000.

Continuous Intraperitoneal Insulin Infusion Pump

Continuous intraperitoneal insulin infusion (CIII) involves surgical implanting a hockey puck-size pump and insulin storage well into the abdominal tissue. A catheter runs from the pump through the peritoneal tissue surrounding the abdominal wall. The insulin reservoir holds enough insulin to last around 2-3 months (Renard, 2002). The well can be refilled at the hospital with a simple skin puncturing procedure. Rates of insulin infusion are controlled using an external programmer that communicates with the pump using radio waves. The benefits of this implanted pump are that it allows for much quicker absorption and action than CSII due to the access through the peritoneum (Selam and Charles, 1990). The persistence of insulin is also reduced which lessens the risk of severe hypoglycemia when compared to CSII (Renard, 2002). However, because this device is implanted, it encounters more issues with biocompatibility. The catheters can become occluded with fibrin tissue and impede the flow of insulin. This has been shown to occur at a rate of 15 per 100 patients each year (Selam, 2001), although newer pump designs allow a relatively simple flushing of the catheter through a built-in side port. As will be discussed in further detail, one of the body's methods of dealing with a chronic intruder such as an implanted device is to encapsulate it with a thick collagen envelope. If this occurs around the intraperitoneal catheter, it will prevent the infusion of insulin and require a laparoscopic debridement in the operating room (Renard, 2002). Due to its effectiveness and "more physiological approach, implantable pumps appear as the most appropriate mode of insulin delivery to be integrated into an artificial ß-cell" (Renard, 2002).

The Inflammatory Response

Before one can realistically contemplate implanting a device into the body, it is prudent to understand how the host will respond to such an intrusion. The inflammatory response is a series of steps that the body takes to deal with trauma or foreign objects in the body. This response can be separated into three stages; initial, intermediate, and late (Gerritsen et al., 1999). Within

seconds of implantation, proteins in the extracellular fluid will aggregate onto the surface of the implant. These proteins, mostly albumin, will develop a monolayer on the implant due to their chemical heterogeneity (Williams, 1987). Although it may seem that these initial proteins could clog the membranes of glucose sensors, studies have shown that post-implantation sensitivities are very similar to pre-implantation values demonstrating that this protein monolayer does not prevent glucose diffusion (Gerritsen et al., 1999). Blood clots will also begin to form around the implant due to the trauma caused by the surgical implantation of the implant. If the device is not secured properly, micromotion will cause additional blood clot formation even after implantation (Koudelka et al., 1991). Blood clots will cause problems for glucose sensors by obstructing the diffusion of glucose to the sensor in addition to limiting the supply of blood in the area for in which glucose may be tested.

The intermediate stage of the inflammatory response can have a strong impact on sensor outputs. Immune system cells such as macrophages and monocytes will attach to the implant and release a barrage of oxygen radicals and proteolytic enzymes in an attempt to destroy the object. These free radicals will lower the pH in the surrounding area which can impact the activity of the enzymes used in the sensor. The physical adhesion of these cells to the sensor can also obstruct the diffusion of glucose which will alter the sensor output. For example, Figure 13 below shows the effect on power density of reducing biofouling on the electrode surface of glucose-based MFCs.

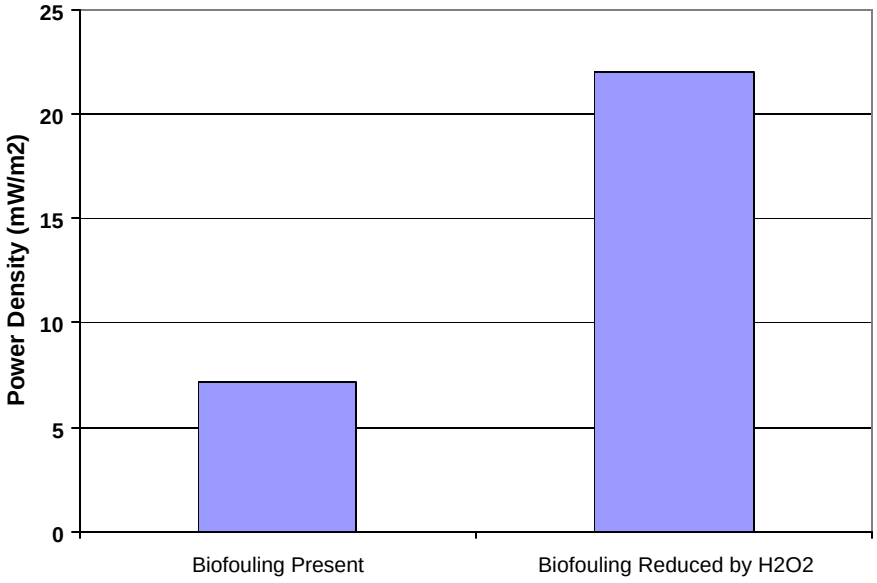

Figure 13: Effect of Biofouling on Power Density (modified from Tartakovsky and Guiot, 2006)

Macrophages and monocytes have been shown to change the oxygen and glucose concentrations in the surrounding extracellular fluid, which will also affect the performance of glucose oxidase in the sensor (Rebrin et al., 1992).

If the initial and intermediate stages of the inflammatory response are not successful in destroying the implant, the body will enter the late stage response. In this stage, the body will attempt to isolate the foreign body by forming a thick collagenous membrane around it. The performance of the device is "dependent on the fibrosity and vascularity of the capsule surrounding the sensor" (Gerritsen et al., 1999). The thickness of this fibrous membrane has been shown to be affected by the chemical and geometrical properties of the implant, mechanical factors at the implant-tissue interface, changes in pH, oxygen levels, and electrical currents produced (Black, 1992). The characteristics of this membrane are highly dependent on each individual patient. If the membrane is highly vascularized, the impact on glucose diffusion may be minimal. However, if the membrane is thick and without much blood supply, the sensor may be completely isolated from the body and will not provide any useful data about blood glucose levels.

Cardiac Pacing

Another potential biomedical application of MFCs is in the area of cardiac care. The power generated from an MFC in the body, regardless of what substrate is used, could be used to power a biomedical device such as a pacemaker. Thus it is important to have a basic understanding of cardiac pacing and devices currently used to treat pacing disorders.

A pacemaker is a device implanted into a patient to assist with cardiac function. Disorders most commonly treated by pacemakers are bradycardia and tachycardia, where the heart is beating too slow or fast, respectively. When the normal cardiac electrical system does not function properly, a pacemaker has the ability to restore a regular beating pattern. Since its first implantation in 1958, patient health has been dramatically improved as evident by the fact that over 600,000 pacemakers are implanted each year across the globe (Mallela et al., 2004). One of the most important characteristics of a pacemaker is that it must be able to function reliably for an

extended period of time. Research into the power supply of a pacemaker has been ongoing since the first device was constructed, and numerous different power sources have been employed. Currently, though, none of the utilized power sources can supply the required power for an unlimited amount of time. Thus a patient must have another surgery to replace the power supply for the pacemaker every few years.

Normal Cardiac Electrical Pathway

The electrical signal for contraction of the heart begins in the sinoatrial node near the top of the right atrium (Figure 14). This signal spreads across both atria causing contraction and movement of blood from the atria to the ventricles. The electrical signal passes through the atrioventricular node, which slows down the signal enough to allow the atria to completely contract prior to ventricular contraction. The signal from the atrioventricular node is passed in the His bundle and then spread through the right and left bundle branches. These branches innervate both ventricles and cause ventricular contraction. This cycle is repeated for every contraction cycle of the heart.

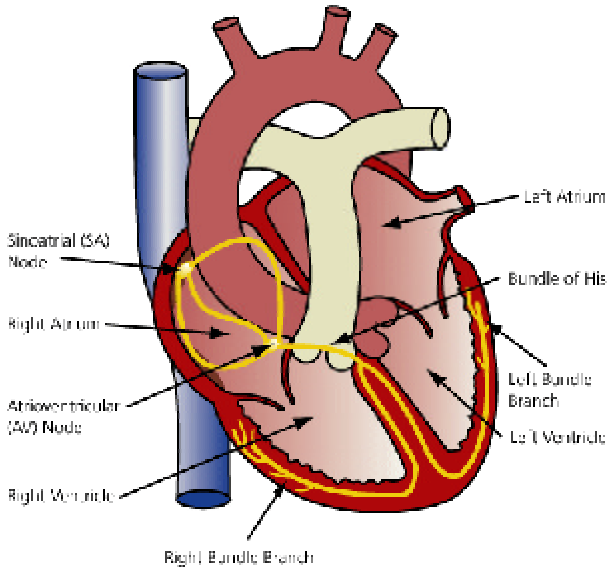

Figure 14: Cardiac Electrical Pathway (St. Jude Medical, 2006)

Pacemaker Function

A pacemaker is comprised of a pulse generator and lead system. The titanium pulse generator "houses electrical components responsible for generating the pulse (via output circuits) at the proper time (via timing and control circuits) based on events sensed (via sensing circuits)"

(Mallela et al., 2004). The generator compartment also contains the battery and any materials necessary for transmitting or storing data. This pulse generator is typically implanted above the pectoral muscle and under the clavicle. Information and electrical signals are transferred between the pulse generator and the cardiac tissue by the lead system. The wires of the lead system attach to the pulse generator and are typically fed through the subclavian vein and into the heart. This system of wires can be either unipolar or bipolar which determines the number or leads running away from the pacemaker. A unipolar pacemaker has one lead that attaches into the wall of the right atrium, while a bipolar unit has an additional lead that attaches into the wall of the right ventricle. Rate responsive pacemakers can sense body movements and breathing patterns to adjust heart rate to a level suitable for the current activity of the patient. Some of the newest lead systems also elute steroids over time to slow the body's natural inflammatory response at the site of electrode attachment (Ellenbogen, 1999).

A pacemaker typically sends a pulse having around 25μJ of energy in 1ms, which is about 2 million times smaller than the energy required for defibrillation (Mallela et al., 2004). In addition to providing the required power, necessary characteristics of the item include reliability, safety, biocompatibility, and a small size/weight. Due to the importance of proper function for patient health, a power supply should be extremely reliable, having no more than 0.005% failures per month (Mallela et al., 2004). Since this device will be implanted, it is important to consider the reaction of the body on the device and the reaction of the device to the body. The power source should preferably be a nontoxic material, so in case of device failure or fracture there is a minimal risk of accidental poisoning. The materials exposed to the body should be biocompatible in order to maximize the operating life of the device. A typical pulse generator has dimensions of 49mm x 46mm x 6mm, weighs around 20 grams, and is shaped as an elliptical object to avoid damage to the surrounding tissues (Mallela et al., 2004).

Pacemaker Power Sources

Numerous different materials have been used for power sources in pacemakers, the vast majority being chemical batteries. These batteries produce power through simple oxidation-reduction reactions occurring at the anode and cathode within the device. The first power source used in 1958 was a nickel-cadmium battery that could be inductively recharged. It maintained its charge

for 4-6 weeks, and it was the responsibility of the patient to recharge the battery. Due to its extremely small capacity and issues with patient noncompliance, this material was quickly abandoned. In the early 1960's, a zinc mercury battery was utilized that was actually composed of several smaller zinc mercury batteries connected in series with one another. This device had a life of two years, which was a significant improvement from the nickel cadmium. However, the main problem that arose with this device was fluid leakage causing power shorts. The 1970s brought about the age of lithium batteries. Numerous compounds of lithium have been used in pacemaker batteries, including lithium-iodide, but all are powered by the oxidation of lithium and reduction of the other compound. This power source is advantageous because the container can be hermetically sealed, preventing fluid or gas leakage into the container. However, sealing the battery causes the byproducts of the oxidation-reduction reactions to be stored in the battery, and increases internal resistance over time. "Beginning-of-life impedance ranges from 50-100 Ohms…and the impedance increases during service to values from 20,000-30,000 Ohms during the accumulation of discharge product" (Mallela et al., 2004). The lithium batteries typically have a useful life of around 10 years, making them the current power source of choice for pacemakers. Nuclear batteries were experimented with in the 1980s and function through heat generation from alpha particle emission by ^{238}P. With a half-life of 87 years, this power source could last well beyond the expected life of the patient. However, problems that arose included travel restrictions (due to state and country regulations for carrying nuclear material), extreme toxicity, and high cost. There have been some interesting theoretical power sources that are currently in research stages, such as biothermal batteries that produce electricity from body heat (Weiner, 2005).

Current Role of Microorganisms in the Body

The average human has over 10^{14} bacteria on the skin, mouth and gastrointestinal tract, compared to 10^{13} human cells. In reality, humans should be thought of as a host-microbial communities rather than individual beings (UW Department Bacteriology, 2006). Bacteria play important roles in our lives, thus the idea of using microbes to generate electricity within the body should not be objectionable. The natural flora of microbes in the mouth is not detrimental to our health, but rather consumes resources so that pathogenic bacteria cannot grow. Microorganisms in the small intestines promote growth and development of the capillary

network and better utilization of food (UW Department of Bacteriology, 2006). In the large intestines, these microbes produce vitamin K and B, which are then absorbed into the bloodstream, and help break down fibrous wastes. In the urogenital tract, these organisms can outgrow bacteria that can cause yeast infections and other disorders. Microorganisms play important parts in maintaining our health, and may be able to help power biomedical devices as well.

Specific Aims

Clearly there is a possibility for microbial fuel cells to be incorporated into biomedical applications. Since these fuel cells have been shown to operate on glucose, two possible biomedical applications are as a glucose sensor or as a power source for pacemakers.

- For glucose sensing, the most important characteristic of the sensor is accuracy. As stated by Renard (2002), "accuracy implies precision, linearity, sensitivity, selectivity, and stability of glucose measurement during *in vivo* use". In order to determine whether an MFC may be a feasible glucose sensor, the following specific aims must be addressed:

    - Is the output of an MFC able to be linearly correlated to glucose concentration?
        - Linear power output of the MFC compared to glucose concentration is important to allow accurate sensing of sugar in the body. If power output were correlated to glucose concentration using an upper order polynomial, the actual glucose levels could not easily be determined from power output. A linear correlation allows minimal computation by the sensor and makes calibration procedures easier as well.

    - Can the output of an MFC be determined in advance based on expected glucose concentration? In other words, how precise is an MFC?
        - The precision of the MFC must be determined before incorporating this technology into glucose sensing. Looking into the future, if MFCs were to be utilized for glucose sensing, mass production of the devices would be necessary. In order to accomplish this, a producer must be able to predict

the power output of the device when under certain conditions simply based on the technical design of the product. In other words, if two identical glucose sensors were developed to sense glucose levels, it would be expected that both sensors report the same power output when testing the same glucose concentrations. One of the problems encountered with Continuous Glucose Monitoring System by Medtronic Minimed (an enzymatic sensor) is that the "sensibility to glucose…is not fully reproducible" (Renard, 2002). A device that lacks precision is not suitable for glucose sensing, thus the precision of an MFC must be determined.

- How sensitive is the MFC? Can the MFC respond to small enough changes in glucose concentration to be useful to a diabetic patient?
  - The typical range of glucose in the bloodstream is 60-140mg/dL (3.3-7.7mmol/L). This range is relatively small meaning that to be applicable to this area, an MFC must be sensitive enough to respond to small changes in glucose concentration. Considering currently used handheld meters are accurate to +/- 10% of actual values in the normal blood sugar range (Saudek et al., 1997), an MFC should be able to distinguish between 5mg/dL changes in glucose concentration at a minimum.

- Is the MFC capable of operating for extended periods of time?
  - Considering that sufficient methods already exist for a diabetic patient to determine their blood sugar on a non-continuous basis, the impetus for incorporating MFC technology is the hope that it can provide a continuous and reliable power output relative to glucose concentration. If the MFC is not capable of operating for extended periods of time, there is much less of a reason to incorporate it into this area.

- Is the MFC selective for glucose?
  - In order to correlate glucose concentration to power output, the MFC must be unaffected by other potential fuel sources in the body, such as acetate,

sucrose, or waste products. Enzymatic sensors are attractive due to their ability to select for glucose. The inability of an MFC to select for glucose will weaken the argument for incorporating it as a glucose sensor.

- Can this device be operated *in vivo*?
  - Current methods such as enzymatic sensors are already able to fill the need for external glucose sensing. In order to incorporate MFC technology to glucose sensing, it must be demonstrated that the device can operate in the internal body environment.

- For the purpose of determining whether an MFC is capable of powering a pacemaker, the specific aims are slightly different. There is less of a need for the MFC to be selective for glucose or to have a linear output compared to glucose concentration, but an increased need to provide a larger power output. Namely, the specific aims to be addressed are:

  - Can the MFC provide enough power for cardiac stimulation?
    - As stated earlier, the power output required for cardiac stimulation is 25µJ in one microsecond (Malella et al., 2004). It must be demonstrated that a fuel cell could provide this amount of energy and yet be small enough in physical size to be implanted.

  - Is the MFC capable of operating for extended periods of time?
    - Considering the ramifications involved for the patient if the power source of a pacemaker were to malfunction, it is critical to demonstrate that an MFC can reliably function for a period of several years, if not indefinitely.

Methods

Experiments and literature review were conducted to provide data for the formulation of answers to the specific aims addressed previously.

Rhodoferax ferrireducens

As discussed earlier, *Rhodoferax ferrireducens* has demonstrated the ability to operate on glucose in a microbial fuel cell, thus attempts were made to produce a viable culture of this organism. A freeze dried culture of *R. ferrireducens* was obtained through the American Type Culture Collection in the fall of 2005. Please refer to Appendix I for propagation procedures used. Following ATCC recommendations, three weeks were allowed for incubation and yielded no microbial growth. A replacement vial of freeze dried culture was sent from ATCC in December 2005 and a second round of propagation took place. At the suggestion of ATCC, 30mM nitrate was substituted for iron(III)NTA in the growth medium used and another three weeks were allowed for incubation. This second propagation trial also yielded no microbial growth. Assistance was provided during propagation from personnel with training in anaerobic technique, and repeated correspondences with ATCC have yielded no answers as to why propagation was unsuccessful. Prior attempts to culture this microbe in our lab in 2004 (Henslee et al., 2004) were unsuccessful which leads one to believe this microbe is simply quite difficult to revive.

Rumen Microbe Experiments

In an effort to secure a viable microbial colony that could operate using glucose in a microbial fuel cell, rumen fluid samples were collected from Waterman dairy farm. Please see Appendix II for rumen fluid collection procedures. Four two-chambered cells previously used in experiments (Henslee et al., 2005) in our lab were set up to operate on the rumen fluid samples. Two glass chambered and two acrylic chambered cells all containing graphite plate anodes, carbon paper with platinum catalyst cathodes, and Ultrex anionic exchange membranes were assembled. Please refer to Appendix V and VI for Ultrex and electrode preparation procedures respectively. Anode compartments were filled with 500mL of rumen fluid and the headspace was flushed with 100% CO_2 prior to capping. Cathodes were filled with 500mL of 0.1M KH_2PO_4 buffer solution and were aerated with oxygen using an air stone. Differential voltage measurements were measured across a 1,000? resistor using a data logger over a period of twenty days. These data are displayed in Figure 15.

Although no growth was evident after twenty days, 200mL of an aqueous solution with varying concentrations of glucose were added to each anode compartment during the first week in January. The amount of glucose added gave each fuel cell a different glucose concentration that corresponded to a realistic glucose concentration found in blood, as shown in the Table 1 below. The voltage data obtained following these additions is shown in Figure 16

	MFC			
	1	2	3	4
Glucose Concentration (mg/dL)	70	110	150	180

Table 1: Glucose Additions to Rumen Fluid MFCs

After allowing these fuel cells to run for another eight days with no change in voltage levels, three new rumen fluid MFCs were started using fresh rumen fluid. The protocols used for collecting rumen fluid and assembling the fuel cells were the same as for the previous trial. The voltage data obtained from this trial are shown in Figure 16.

Dairy Wastewater Microbe Experiments

Following the unsuccessful experiments with rumen microbes and the Ultrex anionic exchange membranes, three new cells were assembled using dairy wastewater as the substrate in early February. Please refer to Appendix III for dairy wastewater collection procedures. Three two-chambered acrylic cells were assembled using graphite plate anodes, carbon paper with platinum catalyst cathodes, and Nafion 117 cation exchange membranes. Please refer to Appendix V and VI for Nafion and electrode preparation procedures respectively. Anode compartments were filled with 500mL of well-shaken dairy wastewater and flushed with 100% CO_2 prior to capping. The cathodes were filled with 500mL of 0.1M KH_2PO_4 and were aerated with oxygen using an air stone. Again, differential voltage measurements were taken across a 1k? resistor using a data logger. The data obtained from this trial are shown in Figure 16.

Following evident growth in the wastewater MFCs, an experiment was done to test the voltage output compared to varying glucose levels. Three new two-chambered acrylic MFCs were assembled with graphite plate anodes, carbon paper with platinum catalyst cathodes, and Nafion 117 cation exchange membranes. The anode compartments were filled with 400mL of a basal growth medium that contained vitamins and minerals necessary for growth. Please refer to Appendix VII for basal medium preparation protocols. In addition to the basal broth, varying

concentrations of glucose were added to each cell, giving final concentrations shown in Table 2 below. Voltage data obtained from this experiment are shown in Figure 16.

	MFC		
	1	2	3
Glucose Concentration (mg/dL)	70	125	180

Table 2: Glucose Concentrations in Dairy Waste MFCs

Glucose Literature Review

To provide more data on glucose based fuel cells, an intense literature review was conducted to find more studies that utilized glucose at levels typically found in the body. Nine studies were found that utilized glucose at normal body concentrations and these data are summarized in Table 3 in the results section.

Results

Rumen Microbe Experiment

The differential voltage values for the first trial of rumen fluid MFCs are expressed in Figure 15 below.

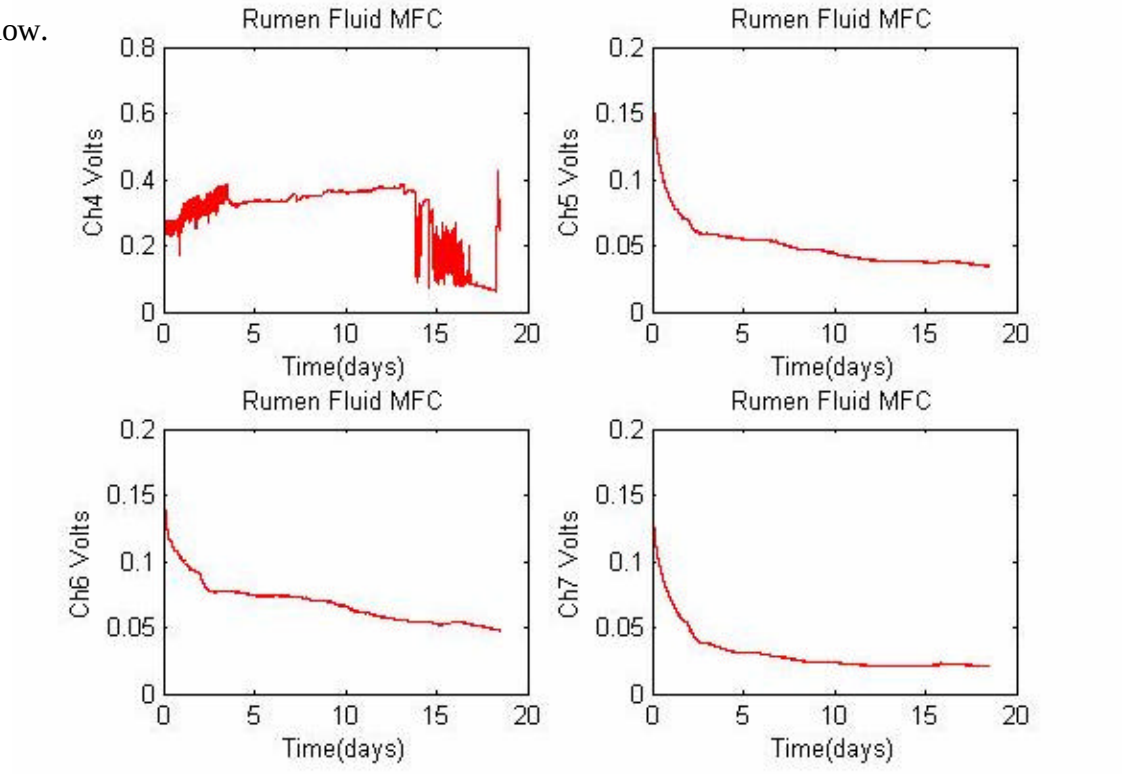

Figure 15: Rumen Fluid MFCs

The initial voltage values expressed in the rumen fluid MFCs are due to a difference in the electrochemical potentials of the anode and cathode. Different chemicals present in the fluid cause a spontaneous oxidation-reduction reaction to occur and give a measurable voltage difference forming a galvanic cell. As these chemicals are degraded, the voltage difference moves to zero. If microbial growth were apparent, there would be an exponential increase in potential difference a few days after the cells are started corresponding to increased microbial activity. No such increases are apparent in this figure, thus it can be concluded that microbial growth did not occur in this trial. The oscillations in MFC 1 (Ch4) are most likely due to problems with the data logger. All wires were cleaned, stripped, and spliced together before each trial to ensure corrosion of the metal wires did not affect measured voltage values. Data logger problems occurred intermittently throughout all stages of the experiment, thus it is most likely the cause of these observed oscillations as well.

Although no growth was apparent in the first trial, glucose was added to each fuel cell in the hope that it would spur microbial activity. This data is shown in Figure 16 below.

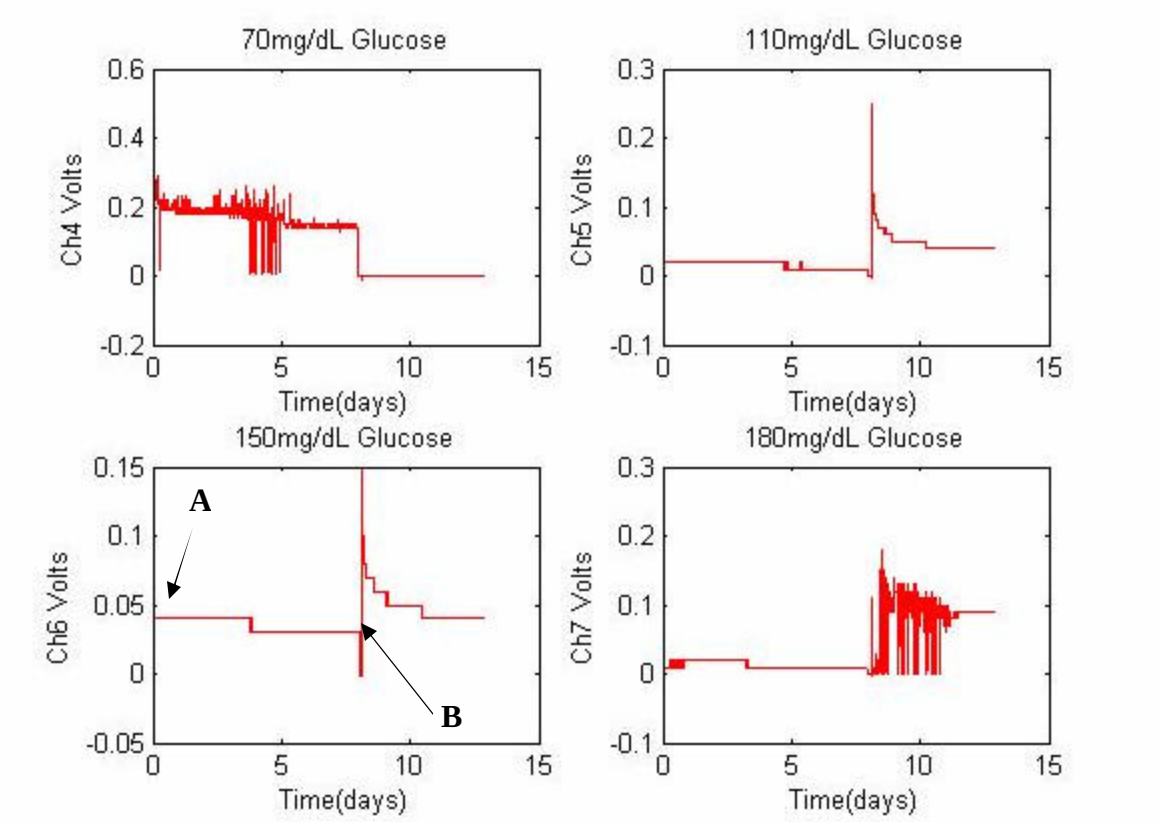

Figure 16: Rumen Fluid MFCs with Glucose (A=Addition of glucose, B=New MFCs started)

Glucose was added at Day 0 (point A) in the graphs above, and as implied by the stagnant voltage differentials, no microbial growth is evident. After eight days with no change in voltage values, these four MFCs were disconnected. The spikes shown around Day 8 (Point B) correspond to the start of three new MFCs with fresh rumen fluid and no glucose in the hopes of obtaining microbial growth. Channel 4 was not connected this time due to the oscillations encountered with the first trial, although as shown by the oscillations in Channel 7 voltage values after Day 8, this problem was not isolated to Channel 4 of the data logger.

Since previous experiments with rumen fluid in our lab have been successful in obtaining microbial growth, I compared my protocol with that of the previous experiments to determine what may have caused the lack of growth. The only difference found is that I used Ultrex *anionic* exchange membranes in these trials rather than Nafion *cationic* exchange membranes. When one considers the mechanism of function for this fuel cell, this difference may have created an incomplete circuit. As the substrate is oxidized in the anode, positive hydrogen ions and electrons are released. The electrons travel into the cathode by way of the wire connection and join with oxygen and hydrogen ions to form water. The positively charged hydrogen ions (*cations*) formed in the anode diffuse through the membrane and into the cathode to complete the electrical circuit. If an *anionic* exchange membrane is used, it will only allow the diffusion of negatively charged ions, thus the protons created in the anode will not be able to flow into the cathode and the circuit will be incomplete. To remedy this problem, all further experiments were performed using Nafion 117 cationic exchange membranes.

Dairy Wastewater Microbe Experiment

Measured voltage differential for the MFCs operating on dairy wastewater are shown in Figure 17 below.

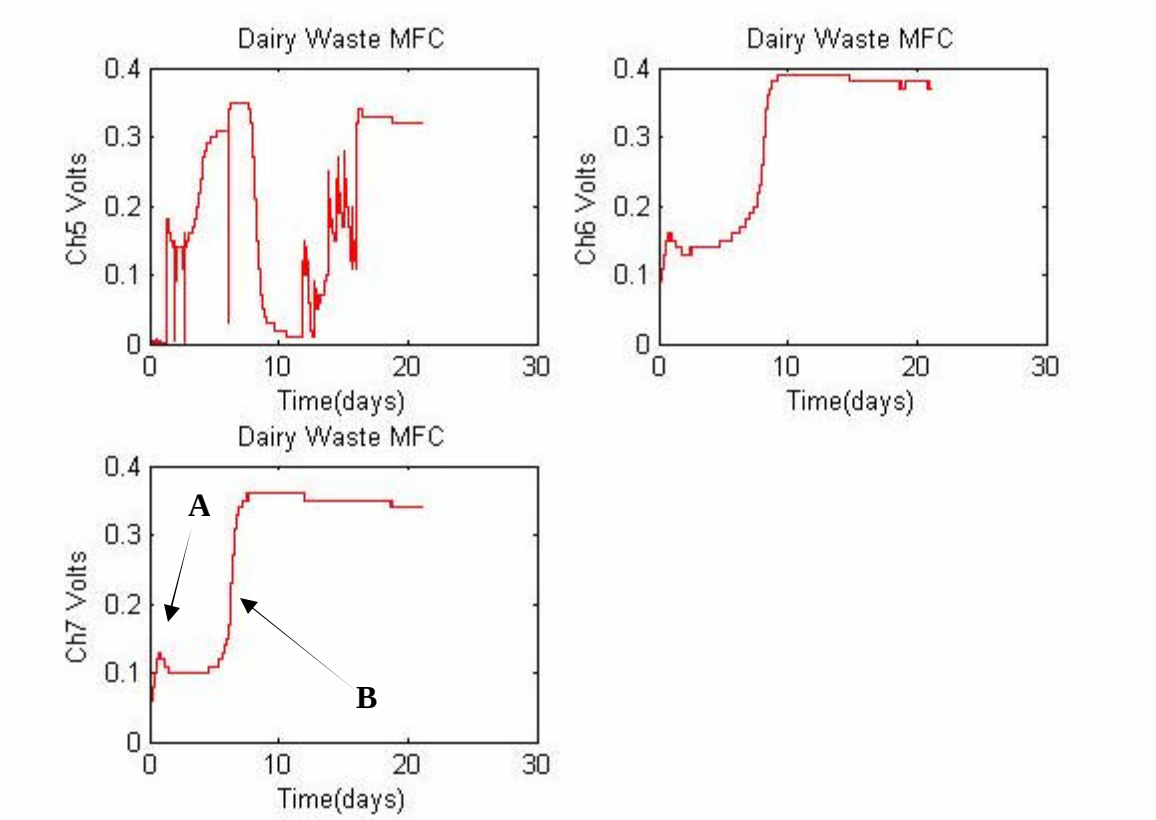

Figure 17: Dairy Waste MFCs (A=Chemical Cell Voltage, B=Microbial Growth Apparent)

In contrast to the rumen fluid MFC experiments, microbial growth is apparent in this trial. The initial spike in voltage values (point A) is due to galvanic cell operation as discussed earlier. The exponential increases in voltage around Day 7 (point B) correspond to microbes colonizing on the electrode surface. As microbes reproduce to yield more microbes, more substrate is oxidized which sends more electrons through the resistor and increases the electrical potential. Day 10 corresponds to steady state being achieved in the microbial colony. Microbes were generating at the same rate that they are dying off. The steady decline after this point corresponds to a faster rate of death than rate of reproduction due to the decreasing amount of resources available.

At this point in the experiment, the electrodes from this trial showing microbial growth were transferred to a growth medium containing various concentrations of glucose. The differential voltage values measured are shown below in Figure 18.

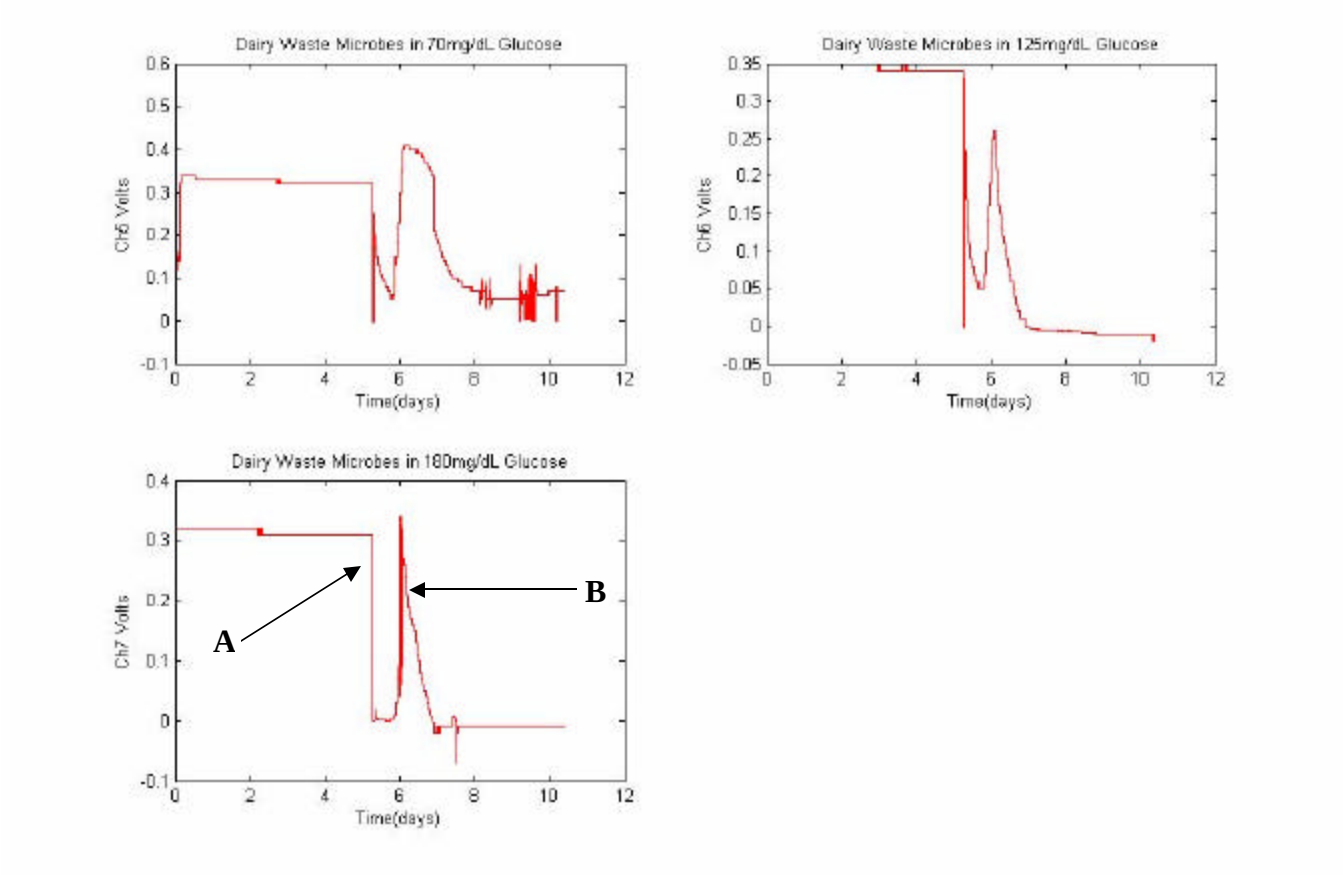

Figure 18: Dairy Waste MFCs with Glucose (A=Electrodes with colonized microbes transferred to glucose broth, B=Acclimated microbes consume glucose in broth)

The drops in voltage at Day 5 (point A) correspond to the time of electrode transfer to the glucose broth solution. It took approximately 24 hours for the microbes to respond to this shock in their environment, but power outputs quickly increased on Day 6 (point B). Power outputs dropped off quickly on Day 7 which probably corresponds to the depletion of glucose in the fuel cells as no other substrates were made available to the organisms in their growth medium. The peak voltages and power densities measured for each glucose concentration are shown in Figure 19 below.

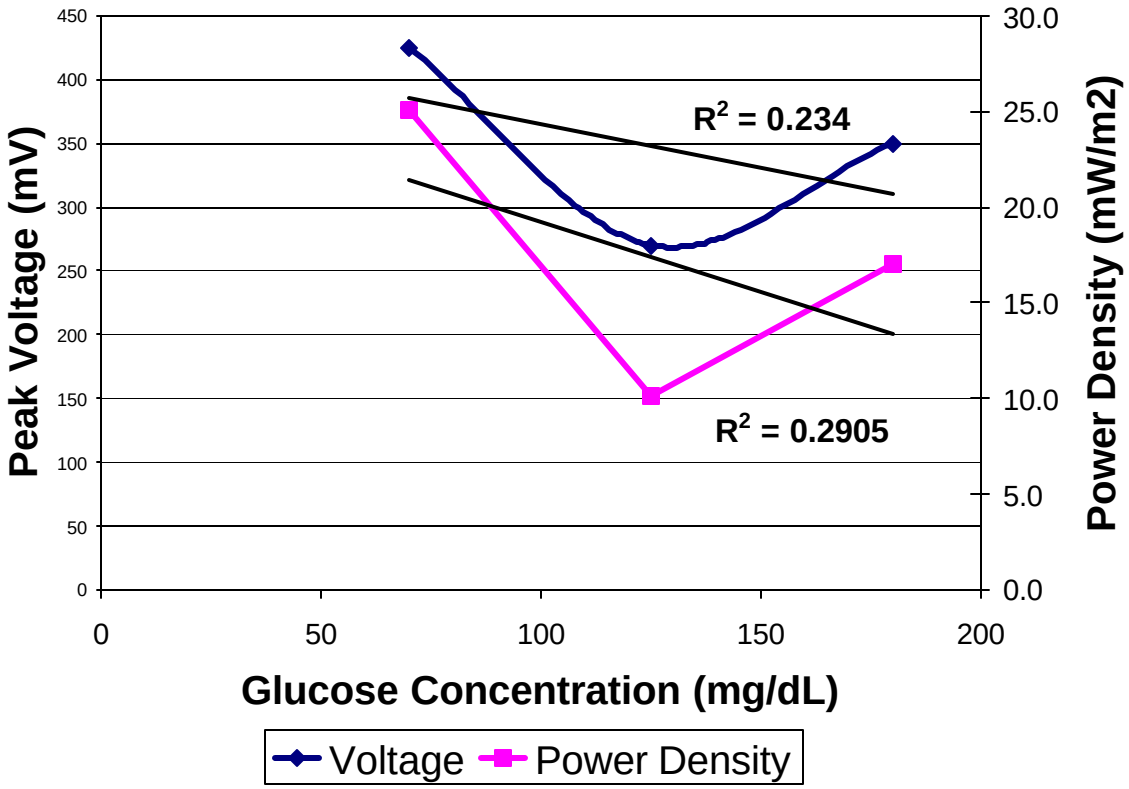

Figure 19: Peak Voltage and Power Densities for Dairy Waste MFCs with Glucose

As shown in this figure, there does not appear to be a good linear correlation between glucose concentration and power density. The likely reason for this is that it is difficult to control the number of microbes colonized on each surface. The more microbes present, the more current can be generated over the same resistance. If a method could be implemented to give the same cell concentrations on each electrode, perhaps a more linear correlation could be drawn. At the same time, perhaps controlling microbe concentrations and exposing them to various glucose levels will only extend the time the cell operates and not give a larger power output. Time constraints prohibited experimentation to explore this area further.

Results from Glucose Literature Data Collection

Data collected from nine studies involving glucose substrates at normal blood-glucose levels are shown below in Table 3. For studies that gave glucose concentrations in terms of COD, the glucose concentration was calculated in mg/dL by the following method.

- Oxidation of Glucose = $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2+6H_2O$
  - MW of glucose = 180g/mol
  - MW of oxygen = 32g/mol
  - COD is the mass of oxygen necessary to completely oxidize a compound

Therefore COD = $(6mol O_2/mol\ C_6H_{12}O_6)*(32g O_2/mol O_2)*(1mol\ C_6H_{12}O_6/180g\ C_6H_{12}O_6) = 1.07g\text{-}O_2/g\text{-}Glucose$

Table 3: Glucose at Body Concentrations Used in Literature

Author	Bacterial Source	Glucose Conc. mg/dL	Waste water COD mg/L	Volts (V)	Current (mA)	Resistance (Ohm)	Power Density mW/m2	Coulombic Efficiency (%)	Chambers	Flow	Cathode Catalyst	Anode Area (cm2)	Anode Material	Membrane
Oh, Logan 2005	Waste Sludge	89.2		0.27	0.6	480	81	27	Double	Batch	Pt, 0.5mg/cm2	22.5	Carbon Paper	Nafion 117
	Waste Sludge	32		0.16	1.25	150	371		Single	Batch	Pt, 0.5mg/cm2	7.1	Carbon Paper	None
Liu, Logan 2004	Waste Sludge		250	0.14		1000	28		Single	Batch	Pt, 0.5mg/cm2	7.1	Carbon Paper	Nafion 117
	Waste Sludge		250	0.33		1000	146		Single	Batch	Pt, 0.35mg/cm2	7.1	Carbon Paper	None
	Waste Sludge	60		0.35		1000	173	48	Single	Batch	Pt, 0.5mg/cm2	7.1	Carbon Paper	Nafion 117
	Waste Sludge	60		0.52		1000	381	10	Single	Batch	Pt, 0.35mg/cm2	7.1	Carbon Paper	None
	Waste Sludge	60			0.92	218	262		Single	Batch	Pt, 0.5mg/cm2	7.1	Carbon Paper	Nafion 117
	Waste Sludge	60			0.86	465	494		Single	Batch	Pt, 0.35mg/cm2	7.1	Carbon Paper	None
Liu, Ramnarayanan 2003							24		Single					

Rabaey, Lissens 2003							3600							
Rabaey, Ossieur 2005		93.4		0.545		100	1179	20	Double	Continuous	Hexacyanoferrate	78.4	Graphite Plate	
			1000	0.445		100	255	14	Double	Continuous	Hexacyanoferrate	78.4	Graphite Plate	
Tartakovsky, Guiot 2006	Waste Sludge	90		0.225	0.4	300	7.2		Double (Upflow)	Continuous	None	118.7	Graphite Rod	None
	Waste Sludge	90		0.35	0.81	300	22		Double (Upflow)	Continuous	H2O2	118.7	Graphite Rod	None
Min, Logan 2004	Waste Sludge		246			470	56		Single	Continuous	Pt, 0.5mg/cm2	100	Carbon Paper	Nafion 117
	Waste Sludge	93.4				320	89		Single	Continuous	Pt, 0.5mg/cm2	100	Carbon Paper	Nafion 117
	Waste Sludge	93.4			0.006	33	220	14	Single	Continuous	Pt, 0.5mg/cm2	100	Carbon Paper	Nafion 117
Rabaey, Clauwaert, 2005	Waste Sludge	46				100	50	43	Single	Continuous	Ferricyanide	V=180 mL	Graphite Granules	Ultrex
	Waste Sludge		33			100	8	22	Single	Continuous	Ferricyanide	V=180 mL	Graphite Granules	Ultrex
Chaudhuri, Lovley 2003	Rhodoferax	36		0.2	0.6	1000	18.5	83	Double	Batch	Ag/AgCl	65	Graphite Rod	Nafion 117
	Rhodoferax	180		0.265	0.2	1000	8.1	81	Double	Batch	Ferricyanide	65	Graphite Rod	Nafion 117
	Rhodoferax	180		0.62	0.57	1000	17.4		Double	Batch	Ferricyanide	20	Graphite Felt	Nafion 117
	Rhodoferax	180		0.445		1000	32.9		Double	Batch	Ferricyanide	65	Graphite Foam	Nafion 117

Figure 20 below shows peak power densities reported in the literature with glucose concentrations at normal blood-glucose levels.

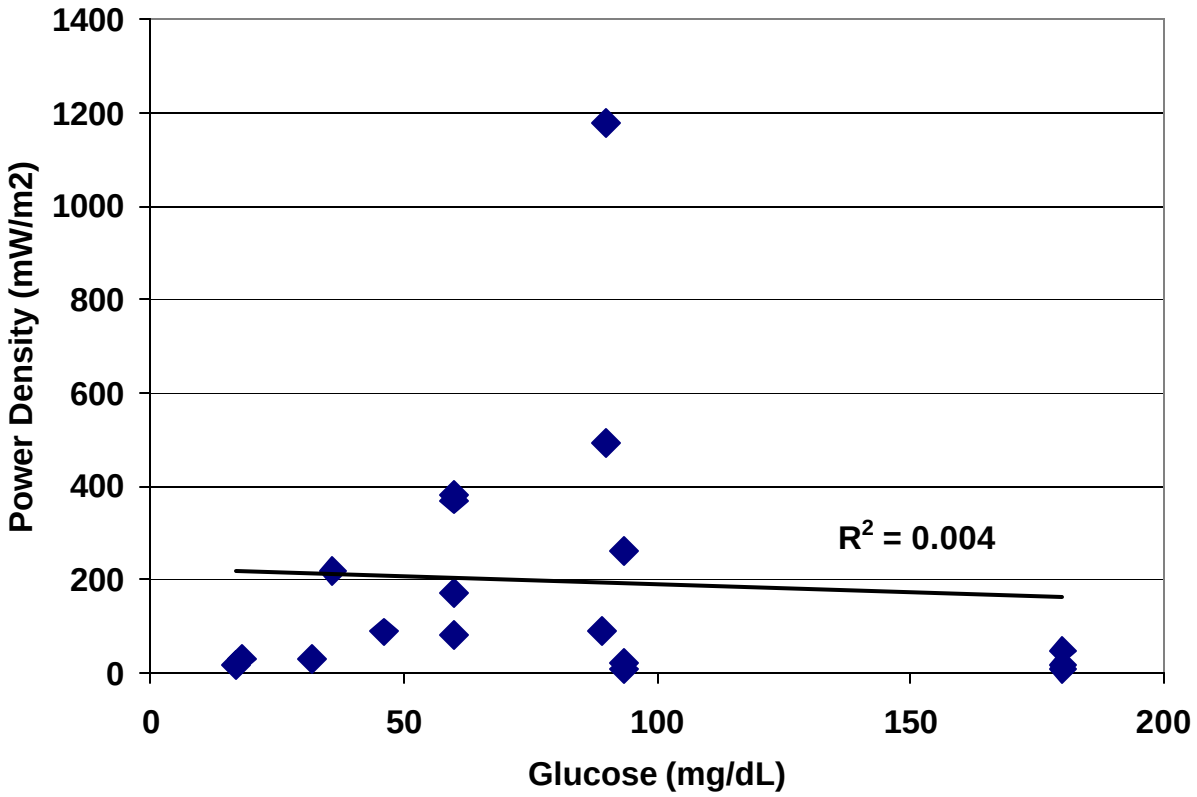

Figure 20: Peak Power Densities vs Glucose from Literature

This graph demonstrates the impact of multiple variables on power output. Considering these values are coming from multiple independent studies, there are several additional variables that are not controlled impacting these data. Such variables include differing electrode materials, cathode environments, and resistance values. For example, the 1200mW/m^2 power density at 100 mg/dL comes from Rabaey and Ossieur (2005) where hexacyanoferrate was used as a mediator in the cathode compartment. This increases the potential between the two compartments and causes a larger power output. This mediator is not applicable to human applications because it is toxic. Valuable information can be drawn from these data, though; it is evident that even when attempting to maximize power density, a linear correlation cannot be drawn between power density and glucose concentration. Also, there is not great precision in the fuel cells as multiple power outputs exist for the same glucose concentrations even within the same studies.

Discussion

Drawing upon the data collected, a discussion of the specific aims set out previously can now be performed.

Applicability of an MFC to glucose sensing

- Is the output of an MFC able to be linearly correlated to glucose concentration?

As shown by the poor R^2 value in Figure 19 and Figure 20 above, the power density of an MFC does not correlate linearly to glucose concentration due to the large number of variables present in the system. If an MFC could be designed so that electrode surface area, oxygen levels, internal resistance, and especially microbe concentrations could be tightly controlled, there may be a linear correlation between glucose concentration and power output. Liu and Logan (2004) studied the maximum voltage as a function of glucose concentration, the results of which are shown in Figure 21 below.

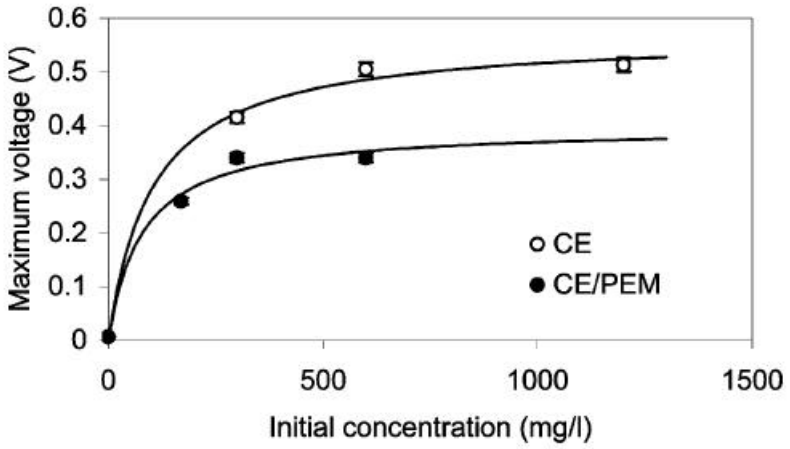

Figure 21: Max Voltage vs Glucose Concentration (Liu and Logan, 2004)

The range of glucose concentrations studied here (0-150mg/dL) correlates well to the expected range of blood-glucose levels, and a linear correlation is clearly not drawn. Power output from this device would therefore also not be linear ($P=V^2/R$) for varying glucose concentrations. The linearity of output from the glucose sensing device is important so that glucose concentrations can be easily calculated by the device. Any other polynomial relationship between output and concentration may have multiple concentrations registering the same output. For example, in Figure 21 above, a voltage output of 350mV could correspond to a glucose concentration of

100mg/dL or 150mg/dL. The treatment protocol for a diabetic patient with either of these glucose levels is entirely different due to the different amount of insulin needed to be injected.

- Can the output of an MFC be determined in advance based on expected glucose concentration? In other words, how precise is an MFC?

Examining Figure 20 above, the conclusion that the precision of an MFC for glucose sensing is not adequate can be drawn. Due to the large number of variables present that impact power output, one cannot readily predict what power output will be obtained from a certain glucose concentration. The only way to determine this power output is to actually do the experiment. Applying this information to glucose sensing, this means that a patient would have to calibrate their device multiple times per day, which is not an improvement over existing glucose sensing methods.

- Can the MFC respond to small enough changes in glucose concentration to be useful to a diabetic patient?

Considering the data presented in Figure 20 above, glucose based MFCs do not appear to be sensitive enough to be applicable to glucose sensing for diabetics. Without a linear correlation between output and concentration, it is difficult to determine the sensitivity as multiple concentrations correspond to the same power output. This can again be attributed to the number of variables affecting power output of the MFC.

- Is the MFC capable of operating for extended periods of time?

The upflow MFC operating on glucose by Tartakovsky and Guiot (2006) was able to operate continuously at steady state voltage for 25 days at which point voltage began to decline, reaching 0V by day 40. After they cleaned the biofouling material from the cathode, an increase in voltage to near steady state value was immediately observed. When they infused hydrogen peroxide into the cathode, the rate of biofouling was reduced and the cell operated at steady state voltage for over 90 days. This demonstrates that an MFC is capable of operating for extended periods of time and likely indefinitely, as long biofouling of the electrodes does not occur. As

discussed earlier, protein fouling of current glucose sensors is a major problem and numerous groups are performing research to reduce this problem. At this point, though, there are no materials that the body will ignore, thus protein fouling will eventually occur. What this demonstrates is that once a method for creating materials that the body will ignore is developed, there is no reason that an MFC with proper filtration could not operate for an extended period of time in the body.

- Is the MFC selective for glucose?

An MFC can be made selective for glucose by two methods: (1) Using a microbe that only consumes glucose, or (2) use filters that allow only glucose to diffuse into the MFC. There are currently no microbes used in MFCs that are selective only for glucose. Microbes that have been shown to use glucose, such as *R. ferrireducens* or mixed cultures from wastewaters, are also capable of operating on simple organic acids and other sugars such as fructose. This implies that for an MFC to be selective to glucose, a filter must be used that is permeable to glucose only. Ward et al. (2002) have reported on filters that are more selectively permeable to glucose; however these filters were only able to be used for 11 days *in vivo* before protein fouling blocked all of the pores. Again, the implication here is that once the materials science field determines how to get the body to ignore materials, it may be possible to filter out only glucose from the bloodstream and thus have a selective MFC.

- Can this device be operated *in vivo*?

As discussed previously, an MFC could theoretically operate *in vivo* (no studies have demonstrated this yet) but it would likely encounter the same problems that current methods of glucose sensing are dealing with. Once the impact of protein fouling and the inflammatory response can be minimized, this device should be able to operate *in vivo.*

Applicability of MFC to cardiac pacing

- Can the MFC provide enough power for cardiac stimulation?

The energy requirement for cardiac stimulation is 25µJ over 1ms according to Malella et al. (2004). Since one joule is equal to one ampere of current traveling over one volt in one second (1J=1A*1V*1s), a unit conversion tells us that 25µJ over 1ms is equal to 0.025W (25mW) over 1ms. Thus, if an MFC is to be able to stimulate the heart, it must be able to deliver 25mW of power. Using the data from Table 3, the surface area of requirements for each fuel cell to provide adequate power for cardiac stimulation is shown below in Table 4. Also shown in Table 4 is the number of carbon paper sheets with dimensions 45mm x 52mm (these are the typical length and width dimensions of a pacemaker according to Malella et al. (2004)) needed to provide the required surface area for cardiac stimulation. Output of 25mW over one carbon paper sheet of typical pacemaker dimensions would require a power density of 10,683.8mW/m^2

Table 4: Required Surface Area for Cardiac Stimulation

Author	Power Density (mW/m2)	Required SA (cm2)	# Sheets
Oh, Logan 2005	81	3086.4	132
	371	673.9	29
Liu, Logan 2004	28	8928.6	382
	146	1712.3	73
	173	1445.1	62
	381	656.2	28
	262	954.2	41
	494	506.1	22
Liu, Ramnarayanan 2003	24	10416.7	445
Rabaey, Lissens 2003	3600	69.4	3
Rabaey, Ossieur 2005	1179	212.0	9
	255	980.4	42
Tartakovsky, Guiot 2006	7.2	34722.2	1484
	22	11363.6	486
Min, Logan 2004	56	4464.3	191
	89	2809.0	120
	220	1136.4	49
Rabaey, Clauwaert, 2005	50	5000.0	214
	8	31250.0	1335
Chaudhuri, Lovley 2003	18.5	13513.5	578
	8.1	30864.2	1319
	17.4	14367.8	614
	32.9	7598.8	325

What this demonstrates is that the power available from an MFC is theoretically enough for cardiac stimulation, but the amount of surface area needed is rather large. To incorporate this much surface area into a standard pacemaker would be quite an engineering feat. Since there is enough power available, some creative solutions for the design of a device to operate *in vivo* will be examined.

- Standard Implantation Above Pectoralis Major

Current pacemaker pulse generators are implanted above pectoralis major and below the clavicle. The subclavian vein runs near this area, and would be able to supply blood to an implanted MFC as well as carry the leads from the pulse generator into the right atrium. The same problems with filtration and biocompatibility discussed in the glucose sensing portion would apply in this case, not to mention the problem of getting enough surface area for adequate power generation. Another problem with this method is that even in the vein, the partial pressure of oxygen is 40mmHg. This oxygen would decrease the efficiency of the system and may prevent it from operating at all, further research would be necessary to confirm this.

- Implantation in Subcutaneous Abdomen

One possibility would be to implant the device under the skin of the abdomen in a similar manner as continuous enzymatic glucose sensors are implanted. Advantages of this idea are that a much larger surface area would be available which would reduce the required depth of the pacemaker to provide the power necessary for cardiac stimulation. For example, if graphite felt, a flexible electrode material, were implanted in an area 6”x6” on the side of the abdomen, the number of sheets necessary for adequate power output would be greatly decreased as shown in Table 5 below.

Table 5: Comparison of Number of Electrodes Needed Per Implantation Site

Author	# Sheets Pectoral	# Sheets Abdomen
Oh, Logan 2005	132	13
	29	3
Liu, Logan 2004	382	38
	73	7
	62	6

	28	3
	41	4
	22	2
Liu, Ramnarayanan 2003	445	45
Rabaey, Lissens 2003	3	0.3
Rabaey, Ossieur 2005	9	1
	42	4
Tartakovsky, Guiot 2006	1484	149
	486	49
Min, Logan 2004	191	19
	120	12
	49	5
Rabaey, Clauwaert, 2005	214	22
	1335	135
Chaudhuri, Lovley 2003	578	58
	1319	133
	614	62
	325	33

There are other additional benefits of subcutaneous implantation of the abdomen. The device will be feeding off of the glucose in the interstitial fluid and not off the glucose in the blood. There will be a smaller filtration requirement in this area, although the same biocompatibility issues will need to be addressed. Perhaps the most important benefit is that, as shown with subcutaneous enzymatic glucose sensors, an oxygen deficit can be created in the skin. This inhibits the effectiveness of enzymatic sensors that rely on oxygen, but it would be a benefit to an MFC as it would increase the efficiency of the electron transport system to have less oxygen present. A mixed culture of microbes could be used to create a more anaerobic environment in this area. Further *in vivo* research would be necessary to confirm this application.

- Intestinal Implantation

Another option for implantation may be to place the anodes into the large intestines. The large intestine is 1.5m long with a diameter of 6.3cm. The main function of the large intestine is to absorb water, absorb vitamin K and B produced by bacteria, and to serve as temporary storage of wastes. With the provided dimensions, the large intestine has a surface area of $0.3 m^2$. An anode constructed of a series of concentric tubes of graphite felt could be inserted into the intestines and anchored into the intestinal wall. The graphite felt is flexible enough that it could fit around the curves of the intestines and not interfere with muscle contraction during bowel movements.

A small cathode could be placed subcutaneously in the abdomen and a wire could connect the anode and cathode together by perforating the bowel. The inferior vena cava runs near the large intestines, and would provide easy access to the right atrium of the heart for pulse delivery.

There are several advantages to implanting in the intestine. The microbial populations in the large intestines are quite large, with over 1,000 different species and the predominant species are strictly anaerobic (UW Department of Bacteriology, 2006). Studies with *E. coli* operating in an MFC showed that without a mediator it was only able to generate 0.30mW/m^2 (Park and Zeikus, 2003). However, when the same authors used a woven graphite material with a bound neutral red mediator, the power output increased to 152.4mW/m^2. These fuel cells were operating on waste sludge in a single chamber batch process. This finding is exciting considering that the *E. coli* used are the same species found in the large intestines. If an anode constructed of the material used in this study were implanted into the colon, no additional microbes would need to be used as the natural flora in the body could provide the electron transfer. The large intestine is already a semi-continuous flow system, thus no additional pumps would be needed. No filtration would be necessary to protect the microbes as they are already able to survive in the intestines without assistance. Also, the bound mediator in this study was found not to leach into solution during application so toxicity should not be a factor (Park and Zeikus, 2003).

Let's examine how the electrode could be designed to fit into the intestines without interfering with the intended functions of the intestines. To minimize the length of the electrode, multiple concentric tubes could be used. Since the intestines have a 6.3cm diameter, six concentric rings with outer diameters of 6cm, 5cm, 4cm, 3cm, 2cm, and 1cm could be fixed to structural supports and anchored into the intestinal wall. The surface area can be doubled if the neutral red is bound to both sides of the woven graphite. This material was shown to produce power output of 152.4mW/m^2 with *E. Coli*, and 25mW are needed for cardiac pacing. Thus the length of tubing necessary to provide this power can be calculated as:

$$152.4\text{mW/m}^2 = 25\text{mW/A}_{\text{tube}}$$

$$152.4\text{mW/m}^2 * 1\text{m}^2/100^2\text{cm}^2 = 25\text{mW}/(2*p*L*(6\text{cm}+5\text{cm}+4\text{cm}+3\text{cm}+2\text{cm}+1\text{cm}))$$

$$L = 25\text{mW}/((152.4\text{mW/m}^2 * 1\text{m}^2/100^2\text{cm}^2)(2* p *21\text{cm}))$$

$$L = 12.43\text{cm}$$

A CAD design of this conceptual electrode is shown below in Figure 22. With this design, the length of the tube in the intestines would only be 8.3% of the length of the intestines. Further study is needed to confirm, but it is likely that the other 91.7% of the intestines could provide enough surface area for adequate absorption of water and nutrients that little impact on the health of the patient would be noticed.

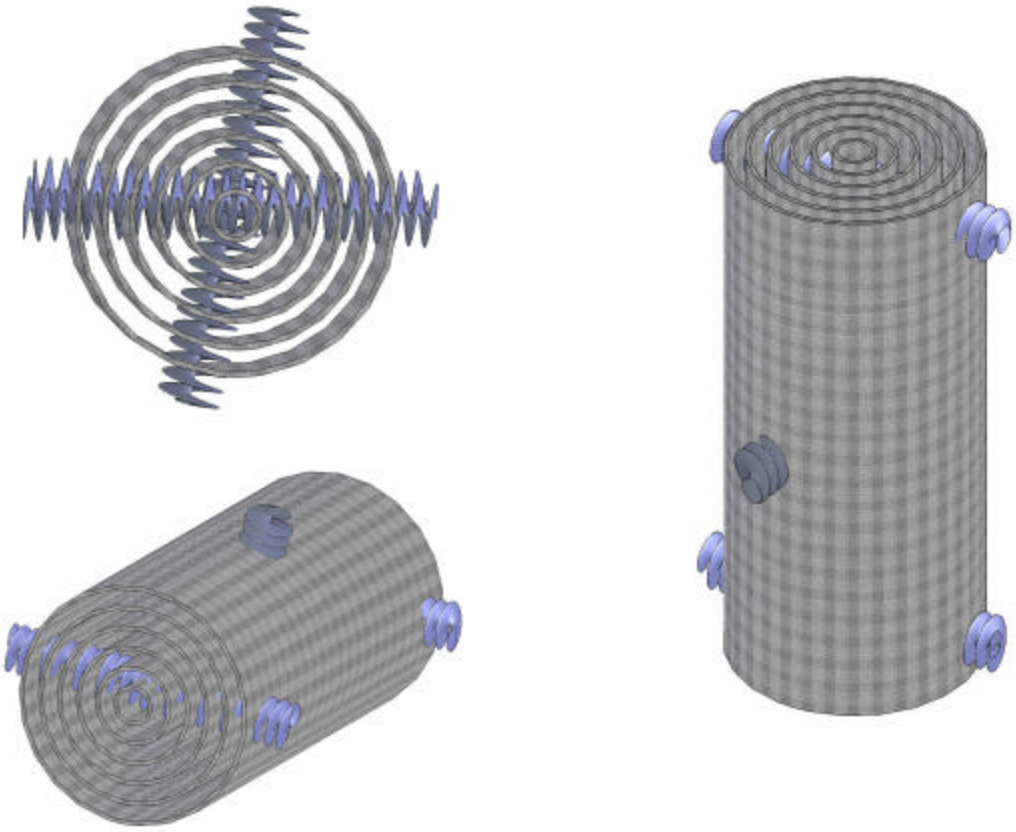

Figure 22: Intestinal Electrode Conceptual Design

The problems that would be encountered in the blood stream due to the high oxygen levels could be avoided by implanting the MFC into the bowel. Another problem that could be avoided is the inflammatory response. The immune system should not attack the fuel cell as long as it is contained within the intestines as the blood supply is present only on the outer coverings of the intestines. The main problem will lie in perforating the bowel. Creating a hole in the bowel now provides an outlet for these opportunistic pathogens to move into other parts of the body. These bacteria are normally not a problem as long as they are contained in the intestine, but small perforations to anchor the anode may cause severe infections of the abdomen. Everything

considered, though, this method for implantation seems to have the most promise for application and warrants further research.

- Is the MFC capable of operating for extended periods of time?

The capability for extended MFC operating life was discussed previously for blood-glucose sensing and the same statements hold true for cardiac pacing. Protein fouling will be the main hurdle to overcome if the MFC were to be implanted above pectoralis major or under the skin of the abdomen. However, the protein content in the large intestines is small as most protein is absorbed in the small intestines. The composition of material in the large intestine is mostly water, indigestible material like cellulose, and dead cells. Fiber in the diet could theoretically help clean the surfaces of the electrodes in addition to providing the fuel source for the microbes. Experiments would need to be done to determine if the electrode would cause bowel impaction. It may be possible to move the electrode to a more proximal location in the bowel, as the content in this section would be more slurry than solid. There does not appear to be a reason why an MFC implanted into the large intestine could not theoretically operate for extended periods of time. In addition, it may be possible to create a hybrid MFC/chemical battery power source. The microbial fuel cell could provide power with a backup chemical battery to ensure dependable cardiac pacing. Since the MFC would be providing the power the majority of the time, the expected life of the chemical battery would likely exceed the necessary operational life of the pacemaker.

Conclusion

Microbial fuel cells represent an exciting new technology in electricity generation and waste treatment. This technology has the potential to offer small amounts of power over near-infinite periods of time due to its reliance on living microorganisms for electricity generation. An established colony of microbes will maintain its population as long as resources are available for consumption, thus continual generation of power is possible. This technology has the potential to allow numerous biomedical devices to operate for much longer periods of time than is currently possible with standard chemical batteries, which would eliminate the need for additional surgeries to replace power sources. In addition, if power output is closely correlated

to fuel source concentration, a real-time glucose sensor could be developed and combined with insulin infusion to form a closed-loop treatment device for millions of diabetics worldwide.

Given the information presented earlier, microbial fuel cell technology has the most potential to impact cardiac pacing power supplies rather than glucose sensing. There are too many variables affecting the power output of a fuel cell that would prevent accurate glucose sensing. However, the amount of power supplied is adequate for cardiac stimulation, and the potential to operate for near-infinite periods of time warrants further research into this application.

Over the course of history, most biomedical devices have attempted to alter the function of the body in some way. Whether it is a total hip replacement that radically changes the joint interface, or an artificial heart that reroutes blood flow, scientists are attempting to re-engineer normal body activities. However, the body is extremely complex and generally finds a way to do what it wants to do. This is evident by the corrosion and loosening of hip replacement implants as well as the protein fouling and membrane formation around artificial heart implants. This response by the body has limited the long term effectiveness of most biomedical implants. Microbial fuel cells have the potential to take biomedical applications in a new direction. Rather than figuring out a way of preventing the body from acting as it normally would in response to a foreign object, the natural processes of the body can be taken advantage of using an MFC. The body naturally maintains a source of anaerobic microorganisms in the large intestines. These same microbes have been able to be utilized in the anode of an MFC. Using these intestinal microbes would eliminate the need to filter substrates in other areas of the body to protect the microbes in the MFC, as utilized microbes are part of the natural flora. The anaerobic nature of the intestines would allow more efficient electricity generation than would be possible in any other area in the body as well. One of the primary purposes of the intestines is for temporary storage of materials. As demonstrated by studies mentioned previously, the power output of a batch MFC is generally higher than a continuous flow MFC. Thus, using the semi-batch process of the intestines would likely provide more power than the continuous flow process of venous blood. Intestinal implantation of an MFC for cardiac pacing represents another method of maximizing the benefits of the symbiotic relationship between humans and microorganisms.

Development of the technology for this application will require a multidisciplinary effort, but the possible benefits warrant the effort.

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Appendix

Rhodoferax ferrireducens Propagation Procedure

Preparation of Basal Medium

- **Ingredients**
  - $NaHCO_3$, 0.5g
  - NH_4Cl, 0.05g
  - $NaH_2PO_4 \cdot H_2O$, 0.12g
  - KCl, 0.02g
  - Wolfe's Mineral Solution, 2mL (ATCC, catalog no. MD-TMS)
  - Wolfe's Vitamin Solution, 2mL (ATCC, catalog no. MD-VS)
  - Distilled Water, 196mL
- **Procedure**

  All basal medium ingredients were combined into a one liter round-bottom flask. Using a Bunsen burner, the contents were brought to boiling. Oxygen was driven out using CO_2 that had been passed through a hot copper column to remove any trace oxygen contaminants. The final pH was adjusted to 6.9 using 1M NaOH. 10mL aliquots of basal medium were distributed amongst 20 25mL test tubes. The headspace of each tube was flushed with CO_2 and then the tube was sealed with a rubber stopper. These filled test tubes were autoclaved for 20 minutes at 121^{oC}

Preparation of 1.0M Acetate Stock Solution

- **Ingredients**
  - $NaHCO_3$, 13.6g
  - Distilled Water, 100mL
- **Procedure**

  The sodium acetate was dissolved into 50mL of distilled water in a round-bottom flask and the solution was brought to a final volume of 100mL. This solution was bubbled with N_2 that had been passed through a hot copper column for 45 minutes.

Preparation of 0.5M Fe(III)NTA Stock Solution

- **Ingredients**
  - $NaHCO_3$, 8.2g
  - Na_3Nitrilotriacetic Acid (NTA), 12.8g
  - $FeCL_3$'$6H_2O$, 13.5g
  - 10M NaOH
  - Distilled Water
- **Procedure**

  The sodium acetate, sodium nitrilotriacetic acid, and iron(III) chloride were combined into a round bottom flask and brought to a final volume of 70mL with distilled water. The solution was stirred using a magnetic stir bar for 15 minutes and the final pH was adjusted to 6.5 using 1M NaOH. This solution was bubbled with N_2 that had been passed through a hot copper column for 45 minutes.

Preparation of Medium Prior to Inoculation

A sterile syringe was equipped with a 0.2µm filter and a sterile needle. 2mL of 1M acetate stock solution was drawn through the filter and into the well of the syringe. The filter and needle were removed and discarded while a new sterile syringe was equipped. This process was repeated with a separate syringe and filter and 4mL of 0.5M Fe(III)NTA were filter sterilized. The top of a sealed 25mL test tube containing the sterile basal medium prepared was passed quickly through the flame of a Bunsen burner. The rubber stopper was removed and CO_2 was continuously flushed through the headspace in the sterile test tube. 0.1mL of the filter sterilized 1M acetate was injected into the 25mL test tube, followed by the addition of 0.2mL of 0.5M Fe(III)NTA. The test tube was sealed with a rubber stopper and again passed quickly through the Bunsen burner. 0.1mL of 1M acetate and 0.2mL of 0.5M Fe(III)NTA were added to the other 19 25mL test tubes of basal medium in this manner. This process gave a final concentration of 10mM for both acetate and Fe(III)NTA in the basal medium.

Propagation Procedure

The vial containing the freeze-dried culture of *Rhodoferax ferrireducens* (ATCC, BAA-621) was opened and the container removed. The tip of the vial was heated in a Bunsen burner and drops of water were squirted onto the hot tip to crack the glass. The tip was struck with a metal file resulting in the fracture of the tip. The insulation was removed and the inner vial was pulled out with forceps. The cotton plug was pulled away. While flushing the headspace with N_2 a small amount of basal medium was added to the vial to dissolve the freeze-dried pellet. $1/5^{th}$ of this solution was transferred to each of five 25mL test tubes containing the growth medium already prepared. These tubes were incubated at 26^oC for two weeks. Growth would have been evident by slight turbidity of the broth.

Rumen Fluid Collection

Rumen fluid samples were collected from dairy cows at the Waterman Dairy Farm. A cannulated cow was chosen and a harness was placed over the head of the animal. The cow was tied to a post to prevent it from wandering during the rumen fluid collection. The cap of the cannula was removed and set aside to allow access to the rumen contents. Fistfuls of rumen contents were removed by gloved hand and placed into a cheese cloth. This cloth was tightly wrapped around the contents and then placed into a funnel. This funnel was set into a collection bottle and the rumen contents were squeezed into the base of the funnel through the cheese cloth. This allowed the fluid to flow through the funnel and into the collection bottle while leaving the miscellaneous debris behind. After all of the fluid was removed from the sample, the excess debris was set aside into a pile. Another fistful of rumen contents were removed from the animal and the process was repeated until approximately two liters of fluid were obtained. At this point, the pile of compressed debris that had been set aside was put back into the rumen of the animal. The cap of the cannula was securely put into place and the animal was untied.

Dairy Wastewater Collection

Wastewater samples were collected from the manure pit at the Waterman Dairy Farm. Prior to collection, the tank was thoroughly stirred by turning on the pump near the pit. A long piece of rope was tied to the handle of a five gallon bucket, and this bucket was lowered into the manure pit. By dragging the bucket through the pit, the bucket was filled and then pulled back to the

opening of the pit. The wastewater was then poured through a screen and into a collection bottle. Approximately two liters of wastewater were collected by this process.

Dairy Wastewater Microbe Basal Medium

- **Ingredients**
  - $NaHCO_3$, 3.0g
  - NH_4Cl, 0.30g
  - $NaH_2PO_4 \cdot H_2O$, 0.72g
  - KCl, 0.12g
  - Wolfe's Mineral Solution, 12mL (ATCC, catalog no. MD-TMS)
  - Wolfe's Vitamin Solution, 12mL (ATCC, catalog no. MD-VS)
  - Distilled Water, 1176 mL
- **Procedure**

  All basal medium ingredients were combined into a one 1.5 liter Erlenmeyer flask. Using a Bunsen burner, the contents were brought to boiling. Oxygen was driven out using CO_2 that had been passed through a hot copper column to remove any trace oxygen contaminants. The final pH was adjusted to 6.9 using 1M NaOH. This flask was sealed with a rubber stopper and metal wire and then autoclaved for 20 minutes at 121^oC

Electrode Preparations

Prior to use in the fuel cells and between trials, all electrodes were thoroughly cleaned with water to remove any excess debris. The electrodes were then soaked in 1M NaOH for one hour followed by 1M HCl for another hour. When not in use, the electrodes were stored in distilled water.

Membrane Preparations

Nafion

New Nafion 117 membranes were prepared prior to use in the fuel cells. A 70/30 solution of distilled water and hydrogen peroxide was prepared and the membranes were placed into the solution. The solution was brought to a boil in a fume hood and monitored for one hour. The membranes were removed from the peroxide solution and then soaked in 0.5M H_2SO_4 for one hour. After soaking, the membranes were placed into distilled water for at least one hour prior to use in the fuel cell.

Ultrex

Ultrex anionic exchange membranes were prepared for use by first cleaning with distilled water. The membranes were then soaked in the 0.1M phosphate buffer solution used in the cathode compartments for at least one hour prior to use.